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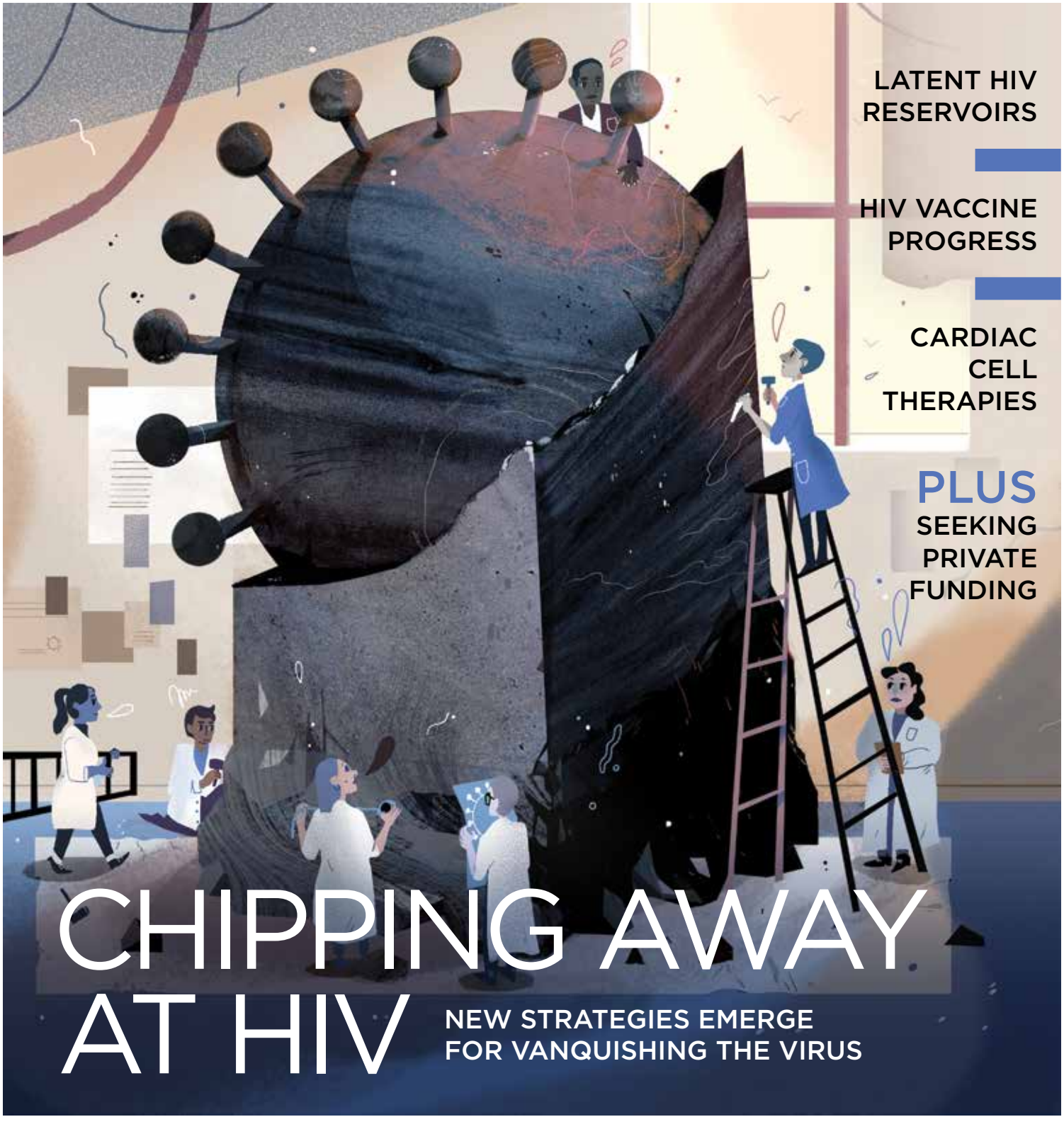

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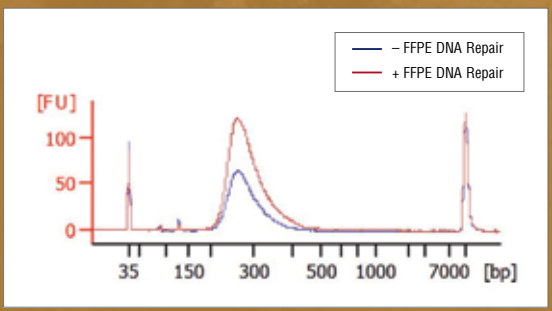


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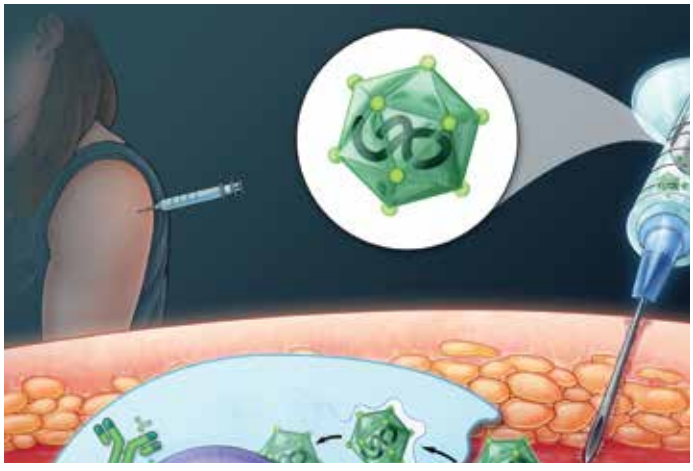

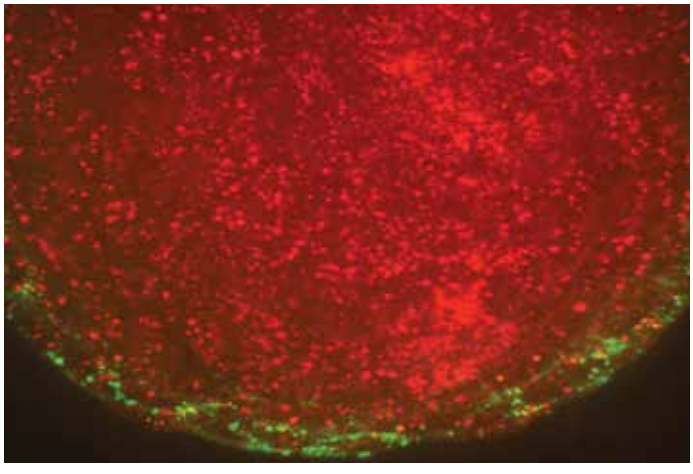

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ON THE COVER: ILLUSTRATION BY MOLLY MENDOZA

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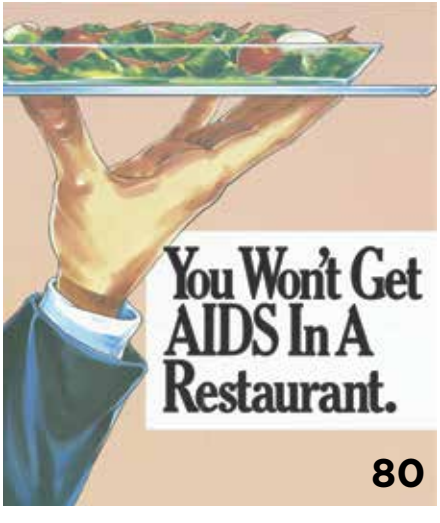




**CORRECTIONS:**
In the "CAR T-Cell Race" (April 2015): the Cellectis IPO date and value was misstated; the company filed an IPO for $228 million in March. In the same article, EP Vantage is the editorial team at the life science market intelligence firm Evaluate Ltd. In "Getting Your Sugar Fix" (April 2015), the number of samples that the Glycosciences Laboratory will analyze for £200 per sample using screening arrays was misstated; the laboratory will analyze as many samples as are necessary with existing probes.

*The Scientist* regrets the errors.

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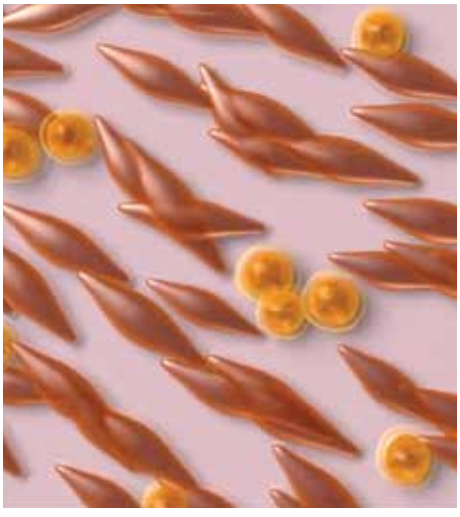


THIS MONTH AT WWW.THE-SCIENTIST.COM:

VIDEO
**Heart Strings**
Watch an animated primer about the harvesting, growth, and administration of cardiac cells to heart attack patients.

VIDEO
**Berlin Activist**
Timothy Ray Brown, the first and only patient to ever be cured of AIDS, is bringing his message of hope to the effort to cure AIDS.

VIDEO
**Eradicating HIV**
Oxford researcher John Frater explains the strategy of targeting latent viral resevoirs to get rid of the virus.

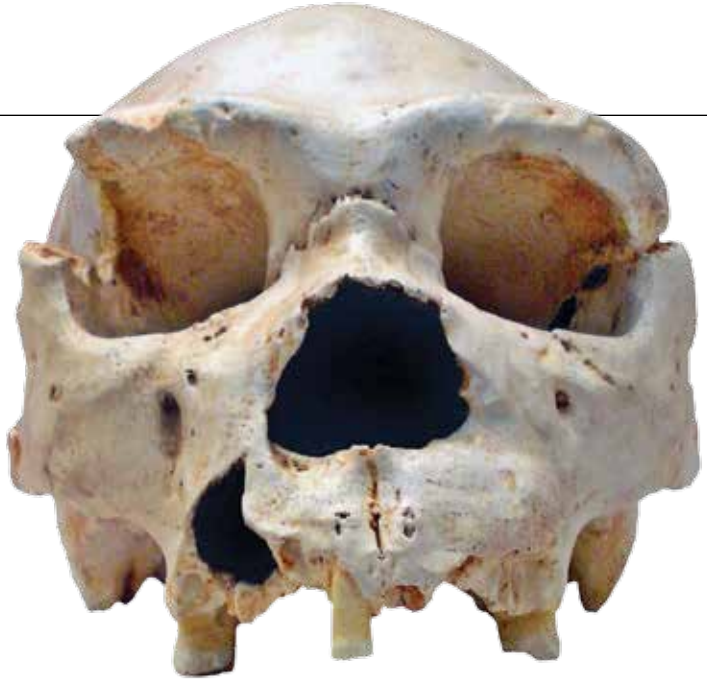
AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

# Coming in June

HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- Advances in the analysis of ancient DNA
- Autocatalytic sets and the origin of life
- Illusions and motion perception
- RNA assays: Seq versus microarrays
- Clinical trial stratification

AND MUCH MORE



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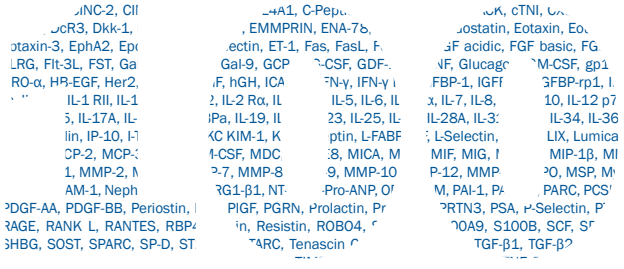


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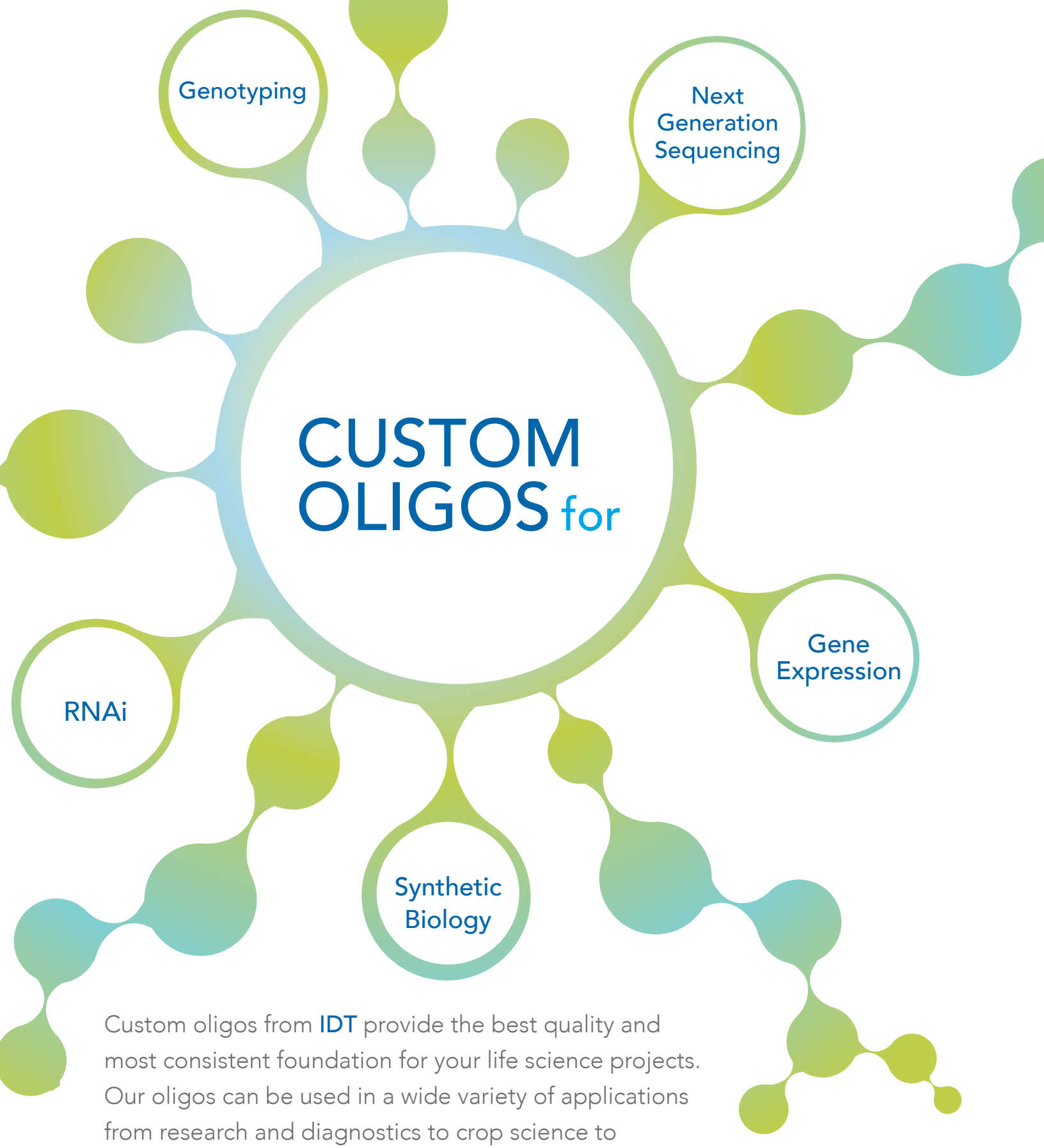


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# Contributors

A native of Melbourne, Australia, **Genevieve Martin** enrolled in a one-year honors program during her medical training at Monash University to study age-related changes in monocytes from HIV-infected women. "When I started this year in HIV medicine, I just became absolutely fascinated, and I thought, this is what I want to do," Martin says. During her honors research, she first encountered the concept of HIV latency and was struck by "the audacity of the idea." Martin has just begun doctoral research on the topic with John Frater at the University of Oxford.

**Matthew Pace**'s high-school fascination with biology continued during his undergraduate years at St. Joseph's University in his hometown of Philadelphia, where he earned extra money over the summer working in a biochemistry research lab. Eventually, Pace became interested in pathogens and disease, and focused on HIV latency during his doctoral work with Una O'Doherty at the University of Pennsylvania. "It's much less straightforward . . . because we don't really understand it yet," he says of HIV latency. He is now a postdoctoral researcher in Frater's lab at Oxford.

Working in the AIDS ward of St. Mary's Hospital in London in the mid-1990s, **John Frater**, fresh out of medical school, decided to conduct HIV research. He pursued doctoral work with Myra McClure at Imperial College London, investigating the responses of different HIV strains to retroviral medicines, and later probed the human immune response to the virus as a clinical lecturer in the lab of Rodney Phillips at Oxford. Frater remains at Oxford, where he is now a research lecturer in the Nuffield Department of Medicine. He is cofounder and codirector of Collaborative HIV Eradication of Reservoirs: UK Biomedical Research Centres (CHERUB), a collaboration among multiple research institutes that seeks a definitive cure for the disease.

Martin, Pace, and Frater explore the mystery of HIV latency in "Hidden Menace," page 34.

---

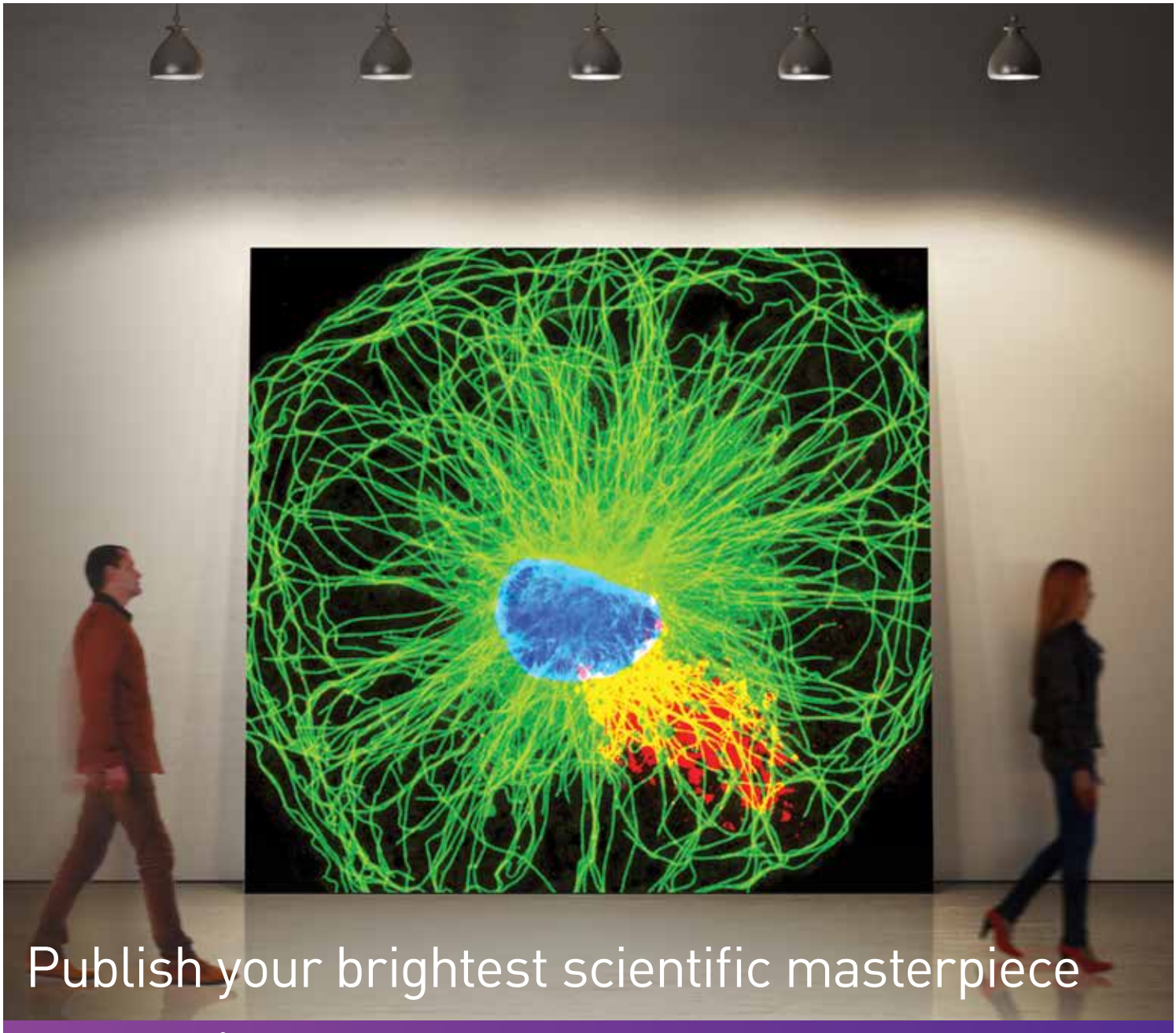
In the 1970s, **Wayne Koff** investigated antiviral drugs against influenza while a doctoral student in Vernon Knight's lab at Baylor College of Medicine. Pursuing what would become a lifelong interest in vaccine development, Koff went on to explore dengue vaccine development and macrophage-mediated suppression of viral infections, a line of inquiry he followed as an assistant professor at the University of Texas MD Anderson Cancer Center in the mid-1980s. In 1986, as the HIV/AIDS epidemic emerged as a public health concern, he undertook a one-year internship at the National Institutes of Health to study the disease, inspiring him to combine his latest research with his long-standing interest in vaccines. After completing his internship, Koff spent four years as NIAID's branch chief for AIDS vaccine research and development before serving six years as vice president for vaccine research and development at United Biomedical. In 1998, Koff joined the International AIDS Vaccine Initiative (IAVI) as vice president of research and development and is today IAVI's chief scientific officer. "We're in a renaissance period in the AIDS vaccine effort," Koff says. "Years ago, people would ask if an AIDS vaccine would ever be possible. Now, the question is no longer 'If.' It's 'When?' "

Koff explains the science behind designing an HIV/AIDS vaccine in "Defeating the Virus," page 40.

---

As a medical student at the University of Ghent in his native Belgium, **Peter Piot** was told there was no future in infectious disease medicine. Undaunted, he conducted doctoral research in microbiology at the Institute of Tropical Medicine Antwerp, where in 1976 he analyzed the blood sample of a Belgian nun who had died in northern Zaire (today Democratic Republic of the Congo). He and his colleagues isolated a completely new and deadly virus: Ebola. In the early 1980s, Piot began serving on the front lines of the rising HIV/AIDS epidemic in both Antwerp's gay community and in Africa among heterosexuals. "I never understood why a virus would care about the sexual orientation of a human host," Piot said in a 2012 interview at the London School of Hygiene and Tropical Medicine, where he has served as director since 2010. Piot's interest in advocacy led him to found and direct UNAIDS from 1995 to 2008. Piot provides his perspective on the history and future of the science and politics of AIDS in an essay, "Attacking AIDS on Many Fronts"(page 74), based on his new book *AIDS: Between Science and Politics.*

GISELLE HABER; JODI GIRNUN; JOHN FRATER; IAVI; HEIDI LARSON

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# Hiding in the Haystack

Encouraging developments in HIV research

BY MARY BETH ABERLIN

Just 10 years after Richard Nixon declared war on cancer in 1971, what appeared to be an anomalous epidemiological puzzle heralded the onset of a new war that continues to be fought against another wily foe, the retrovirus HIV. In the war on cancer, there seems to be a real feeling that some kind of corner has been turned, and last month we focused on some of those hopeful advances, especially in the field of personalized drug regimens and immunotherapy. This month *The Scientist* covers the latest reconnaissance and tactical maneuvers that are exciting hopes that HIV/AIDS can actually be vanquished.

HIV is a stealthy interloper that inserts its genome into the DNA of the T cells it infects, causing a devastating illness. A cocktail of antiretroviral therapies (ART) has changed AIDS from a death sentence into a chronic, treatable condition for many. But the treatment is not a cure. Stop the drugs, and the virus roars back to fight anew. The largest obstacle to ridding the body of HIV is the virus's seemingly universal establishment of latent reservoirs capable of ramping up to produce new infectious virions.

**Hopeful straws in the wind include new vaccine designs and latent HIV eradication.**

Several articles in the May issue address this sneaky behavior. "Hidden Menace" (page 34) explores what's known about the problem and the latest advances being made in the effort to destroy the reservoir. One audacious method, labeled "shock and kill," proposes to reactivate latent HIV in order to wipe it out. On page 54, you can read about recent research aimed at figuring out whether clonal T cells are harboring latent viral DNA. And Modus Operandi (page 33) describes the use of whole-body immunoPET scans to locate tissue areas that host replicating SIV virus in macaques, including in animals known as elite controllers that naturally suppress infection.

Only 14 million of the 35 million people infected with HIV are on ART, according to a World Health Organization estimate. Many of those are not on the treatment because they are unaware that they are infected, and thus serve as sources of new infections. The quest for an effective vaccine that would protect against HIV has been ongoing for as long as the cause of AIDS has been known. In "Defeating the Virus" (page 40), veteran quester Wayne Koff, chief scientific officer at the International AIDS Vaccine Initiative, describes the exciting state of current HIV vaccine research, from designer immunogens based on new knowledge of the most effective broadly neutralizing antibodies, to direct injection of such antibodies (or the genes coding for them), to cellular immunity ramped up via custom-designed mosaic antigens.

New and unconventional drug design continues at a furious pace. A February 2014 online *Nature* paper generated a huge and hopeful buzz, reporting the effectiveness (in animals) of a two-armed decoy made from pieces of the two receptors (CD4 and CCR5) to which HIV must bind in order to gain entry to host T cells. Researchers are investigating unusual antibodies produced by llamas, alpacas, and camels (page 17) for effectiveness as vaginal microbicides because the molecules are relatively insensitive to pH. And CRISPR gene-editing systems designed to cripple CCR5 or to cut out inserted viral DNA have been demonstrated in vitro. Our May profilee, virologist Carol Carter (page 56), studies HIV assembly and release with the aim of identifying new viral protein targets.

There are, of course, many facets other than research that must be addressed if the AIDS epidemic is to be halted. But a view from the ramparts suggests that after more than three decades of relentless basic research, a number of clever flanking actions may finally turn the tide in the war against HIV. ■

Editor-in-Chief
eic@the-scientist.com

ANDRZEJ KRAUZE

# Speaking of Science

**A fatal disease has been tamed into a chronic condition. The next step is to find a cure. Scientists are innately cautious, and AIDS researchers have learned humility over the years. Science operates around a core of uncertainty, within which lie setbacks, but also hope.**

—Harvard Medical School researcher **Jerome Groopman**, in a *New Yorker* article about the quest to find a cure for AIDS (December 22 & 29, 2014)


For a long time scientists were cautious about referring to a cure. The possibility just seemed so out of reach. But people are talking about it more now than they used to. Several drug companies are shifting away from developing new HIV drugs to pursuing strategies for using existing drugs to eradicate the virus.

—**Robert Siliciano**, Johns Hopkins University infectious disease researcher who studies HIV latency, on the website of the university's Institute for Basic Biomedical Sciences

As HIV investigators continue the campaign to control and conceivably eradicate HIV worldwide, certain directions reflecting myths or misconceptions about this infectious disease have been embraced, while other concepts with merit have been left relatively unexplored. These issues in HIV pathogenesis threaten to prevent a true long-term solution to HIV infection and, perhaps, infections by other human pathogens.

—Prominent HIV researcher **Jay Levy** of the University of California, San Francisco, in a recently published opinion article about HIV research (*Trends in Molecular Medicine*, April 14)

The currency of science is fragile, and allowing counterfeiters, fraudsters, bunko artists, scammers, and cheats to continue to operate with abandon in the publishing realm is unacceptable.

—New York University bioethicist **Arthur Caplan**, in a *Mayo Clinic Proceedings* commentary about problems in science publishing (April 3)

I think we're going to have strong indications of life beyond Earth within a decade, and I think we're going to have definitive evidence within 20 to 30 years.

—**Ellen Stofan**, NASA chief scientist, speaking during a recent NASA panel discussion about the search for water in the universe (April 7)

Most work going on in brain imaging is of no scientific value.

—**Margaret Boden**, cognitive scientist at the University of Sussex, U.K., speaking with *New Scientist* about understanding human consciousness (March 31)


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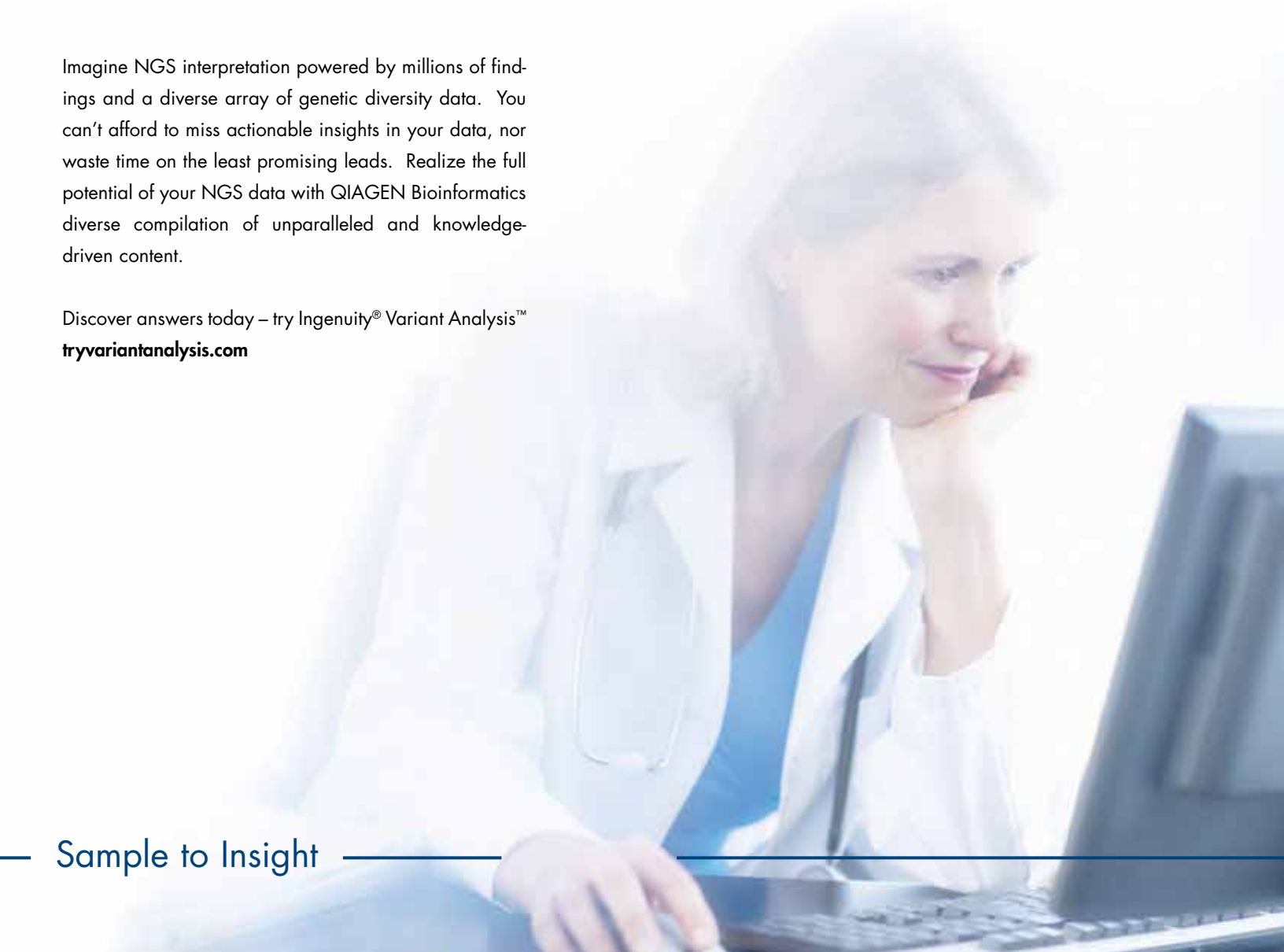

Sample to Insight

# Notebook



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**NO PROB-LLAMA:** Llamas, camels, alpacas, and related animals produce antibodies that are smaller, more heat stable, and more insensitive to pH than typical mammalian counterparts, making them good potential candidates for HIV vaccine design.

## Llamas as Lab Rats

In 1990, Dutch molecular biologist Theo Verrips began hunting for a new toothpaste formula. Having been one of the developers of the Clearblue pregnancy test, Verrips was already an antibody expert, so his superiors at Unilever asked him to add antibodies to toothpaste, where the molecules might be able to incapacitate harmful oral bacteria without contributing to growing antibiotic resistance. He and his colleagues managed to mix effective antibodies into the toothpaste, but it was unfortunately too expensive to manufacture on a commercial scale. Verrips convinced his bosses to let him experiment for one more year by telling them about a new antibody discovery.

The breakthrough Verrips mentioned was the 1989 discovery of a naturally small antibody. Students at the Free University of Brussels, reluctant to purify antibodies from human blood due to fears about HIV, had identified what they thought were antibody fragments in a camel blood sample that had been forgotten in a lab freezer. A professor at the university, Raymond Hamers, and his colleagues were intrigued and demonstrated that the antibodies, found in camels, llamas, and alpacas, were not fragmented, but rather consisted of two heavy chains, instead of the two heavy and two light chains that make up the antibodies produced by humans and many other animals. These unique antibodies account for 70 percent of the antibodies in a camel's blood and 30 to 40 percent in a llama's, says Mehdi Arbabi Ghahroudi of the National Research Council (NRC) in Canada who helped to characterize the antibodies during his doctoral work. Researchers have since tended to study llamas, however, because they are smaller and thus easier to work with.

Heavy-chain antibodies have many advantages. "They're less-bulky proteins and fit into crevices that whole antibod-

ies don't see," says Robin Weiss of University College London who has been working with heavy-chain antibodies for 12 years. Because they can be encoded by a single gene, they are easier to clone, produce, and test in the lab than traditional antibodies. Even without special purification and preparation, the llama antibodies are of higher quality than those from other animals, adds Mohamed El Khattabi, chief scientific officer at QVQ, the antibody-focused biotech that Verrips founded in 2010. Llama and camel antibodies are also heat-stable and relatively insensitive to pH. "They're very hardy little molecules as far as proteins go," Weiss says.

With these potential advantages in mind, Verrips invited Hamers to collaborate in 1994. Despite all those positive attributes, the llama antibodies were still too expensive to include in *Streptococcus*-fighting toothpaste or antidandruff shampoo. Verrips retired from Unilever in 2002 but continued to work with the molecules at the University of Utrecht, where he used llama antibodies to develop successful rotavirus treatments before Weiss approached him about developing llama antibodies against HIV.

In 2006, as the collaboration between Weiss and Verrips began, El Khattabi, then a postdoctoral researcher at Utrecht University, was responsible for immunizing the animals, because he had already learned how to wrangle them by grabbing their ears. He injected the llamas with DNA encoding an HIV envelope protein and twice more with additional envelope components at two-week intervals, in the chest or the hind legs, where the otherwise very thin animals have the thickest muscle. Weiss, his students, and postdoctoral researcher Laura McCoy, now at the Scripps Research Institute in La Jolla, California, then screened the blood for antibodies. McCoy improved initial screens by developing ways to identify broadly neutralizing antibodies, which bind to a wide variety of HIV molecules, regardless of strain.

Broadly neutralizing antibodies are seen as the key to combating rapidly mutating foes such as HIV. (See "Defeating the Virus" on page 40.) The problem is that these antibodies only occur in about 20 to 30 percent of people infected with HIV, and can take two to four years to develop, says pathologist Neil Greenspan of Case Western University who was not involved in the llama research. McCoy, Weiss, and their colleagues first described these llama-derived antibodies in 2012, and in a December 2014 paper reported that multiple immunized llamas could make broadly neutralizing antibodies against HIV (*PLOS Pathogens*, 10: e1004552, 2014). Generating these powerful reagents by immunization rather than infection could help design a better HIV vaccine. "We now know there is a vertebrate immune system that can reproducibly produce the kind of anti-HIV antibodies we need," McCoy wrote in an e-mail.

Verrips and others who work with llama antibodies are excited about the many potential applications of the molecules in the treatment and diagnosis of HIV and other diseases. "Llama antibodies are very good to combat viruses in general, not just HIV," Verrips says. Greenspan says it could be difficult to use llama antibodies directly, as they would have to be adjusted to prevent a human immune response to the foreign protein, but Arbabi says the mol-

**LLAMAS AGAINST AIDS:** A 3-D model of an anti-HIV llama antibody

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**Llama antibodies are very good to combat viruses in general, not just HIV.**

—Theo Verrips, University of Utrecht

ecules are in fact quite similar to human heavy chains.

Already, llama antibodies have improved HIV structural studies by binding to the virus and thus making it more rigid and easier to crystallize. Other applications for the antibodies include using them as imaging agents, including two designed by Verrips and his colleagues to detect breast cancer that will be tested in the clinic this year; as therapeutic agents that can cross the blood-brain barrier; as a lung cancer treatment, L-DOS47, developed by Arbabi and colleagues at the NRC; and as a quick and PCR-free HIV diagnostic test that is being developed by British researchers.

Weiss and Verrips say they're most excited about the llama antibodies' potential as a vaginal gel microbicide to prevent HIV infection during sex. The antibodies' relative pH insensitivity could be useful in maintaining the gel's efficacy in both the acidic vagina and alkaline ejaculate. Researchers in France are currently testing the gel in animals. Weiss hopes that these and other experiments will maintain the research community's interest in the many fascinating and useful properties of llama antibodies. "Theo and I are . . . hoping a new generation of scientists will pick this up and run with it," he says.

—Jenny Rood

# A Most Kinky Moth

Sporting brown wings flecked with white, the gold swift moth looks pretty drab. Its sex life, however, is anything but. Mating in most moth species occurs when the alluring scent of a female causes a male to fly in search of a potential partner. But male gold swift moths that are ready to mate form a large group called a lek from which the females choose their partners.

In 2007, at a summer holiday cottage outside the town of Inverness in Scotland, newly retired University of Leeds entomologist John Turner was washing up the supper dishes as the sun was setting. Looking out into the small clearing behind the house, Turner noticed gold swift moths, a species he had studied previously, flying and mating in the twilight. Abandoning his chore, he rushed outside to watch the moths. He noted the lekking behavior, but he also observed any number of variations: males approaching large groups of females and even two moths meeting and copulating midair.

"They seemed to display every conceivable mating procedure," Turner says.

Each day thereafter, he returned to the stand of woodrush and low-lying plants an hour or two before sunset to watch the moths perform their mating dances, the entomologist armed with little more than a notebook in which he frantically scribbled his observations in the rapidly disappearing daylight. Turner observed the males hovering in midair,

ANDRZEJ KRAUZE

releasing a pheromone that smelled of ripe pineapple, as females flocked nearby. When a female selected a male, the two would retreat to a nearby branch to mate. Even as his retirement continued, Turner was able to get the funds needed to purchase an infrared camera to document the behaviors in greater detail.

In addition to the lekking behavior that he had previously documented, he observed females hanging from leaves and emitting pheromones to attract males. On other occasions, males formed a swarm and followed an individual female until she landed on a leaf, where she proceeded to mate with one of the males. Males and females also appeared to attract each other and dance in midair before mating. A few times, males even tried to mate with each other. The moths' sexual positions were just as varied.

Turner returned each summer for seven years to document the moths' mating behavior. His discoveries, published earlier this year in the *Biological Journal of the Linnean Society*, show that gold swift moths could teach even the authors of the Kama Sutra a thing or two about sexual behaviors.

"Sex is obviously very exciting in the gold swift world," says James Mallet, an entomologist at Harvard University. "We've overestimated the uniformity of sexual behavior when obviously it's quite diverse."

Gold swift moths are common across continental Europe and Great Britain, and naturalists have been studying these nocturnal insects for more than a century. Nearly 40 years ago, one of Turner's earliest papers determined that, while female moths might hover around the males, they didn't knock them out of the air during the mating process, as moth collectors had claimed. But because most insects have fairly stereotypical sexual behaviors that don't vary much, Turner didn't bother to investigate the question much further—until his vacation in the summer of 2007.

Thomas Simonsen, an entomologist at the Natural History Museum, London, says that the varied behaviors of the gold swift moth also contain hints as to how these behaviors might have evolved and how some groups of moths may have made the transition from "typical" moth mating behaviors to lekking, which has only been observed in a few families.

"In moths like the gold swift, the females are actually attracted to the male, but for most other moths and butterflies, it's the other way around," Simonsen says. "By showing how this one moth species displays all of these different mating behaviors in one place at the same time, you can see how this could have evolved." The question then remains; Why didn't scientists notice the gold swift moth's impressive mating repertoire sooner? To Turner, the answer is obvious. Mat-

ing occurs at twilight, when daytime predators have finished hunting but bats and other nocturnal threats haven't yet emerged. But it means that scientists aren't out looking for the moths, either.

"At twilight, none of the moth people are out yet, and all the butterfly people have gone to the pub," Turner says.

—Carrie Arnold

# HIV in the Internet Age

Hooking up has never been easier. For those who want to avoid the crowded, boozy bar scene, finding a sexual partner is just a click away online. One popular site for such cyber-assisted trysts is Craigslist, which has an entire section dedicated to "casual encounters," categorized by who's looking for whom. Also, a number of new smartphone apps have hit the market recently, bringing no-strings-attached sex to the palm of one's hand.

**In the age of social media, new social routes of HIV transmission are possible.**

—Jason Chan, University of Minnesota

With sex so much easier to come by, many researchers and activists have raised concerns about technology increasing the spread of sexually transmitted diseases, such as HIV/AIDS. Social-networking technologies seem especially popular among gay and bisexual men, particularly African Americans and Latinos, some of the most at-risk populations for HIV/AIDS in the United States. Some epidemiological studies have found correlations between the use of such technologies and rates of HIV infection. One study that made headlines earlier this year, for example, found that the appearance of Craigslist in a given region was associated with a 15.9 percent increase in HIV incidence, and that more than 6,000 new cases of HIV infection could likely be attributed to the site each year (*MIS Quarterly*, December 2014).

"The take-away message is that in the age of social media, new social routes of HIV transmission are possible," study coauthor Jason Chan, now at the University of Minnesota, told *The Scientist* in an e-mail. "On the users' end, the adage 'buyers beware' is still very applicable in this context. . . . It would be fruitful for healthcare practitioners and academics to look into these new avenues to uncover areas that are in need of intervention."

But the relationship between social-networking technologies and HIV risk is not straightforward. First of all, it is still unclear whether the correlations that have been documented between such technologies and HIV incidence are indicative of causality, notes Ian Holloway, a social behavioral scientist at the University of California, Los Angeles's Center for HIV Intervention, Prevention, and Treatment Services. Perhaps the technologies are increasing HIV risk, or perhaps they are simply attracting those who tend to engage in sexually risky behaviors. "Really, the question of whether it's selection or influence that's contributing to greater HIV risk behavior among guys who use these platforms is unknown," says Holloway. "There haven't been good longitudinal studies to tease out which of those mechanisms is in place."

Another consideration is what types of sexual behaviors tend to result from Internet hookups. UCLA's Sean Young, a faculty leader of the new UC Institute for Prediction Technology, and his colleagues have found, for example, that men who find male sexual partners on social-networking sites are more likely to have oral sex, which carries a much lower chance of disease transmission (*PLOS ONE*, 8:e62271, 2013). "If it's easier to find people to have sex with, then it definitely increases your risk for HIV just by having a larger number of sexual partners," Young says. "[But] that increase in sexual partners may be mitigated . . . by a change in sexual-risk behaviors, like oral sex compared to unprotected anal sex."

CHAT ROULETTE?: Some studies have found that the appearance of sites such as Craigslist in a given region correlate with increasing HIV incidence.

People looking for sexual partners online may also be able to lessen their risk of infection by having access to information they wouldn't necessarily have about someone they met in a bar. Many technologies geared specifically to arranging sexual encounters even provide users with an opportunity to indicate their HIV status. If honestly reported, this can result in sero-sorting, allowing people to choose a sexual partner who matches their own HIV status. "You have to have a sero-discordant sexual act in order to transmit a new case of HIV," says behavioral health scientist Kathryn Muessig of the Gillings School of Global Public Health at the University of North Carolina at Chapel Hill. "If both people accurately know their status, it could reduce risk."

Independent of how social-networking sites influence sexual-risk behaviors, there

is evidence that these technologies are effective at spreading HIV/AIDS awareness, prevention measures, and even HIV testing. For example, the Harnessing Online Peer Education (HOPE) study, led by Young, found that people who joined Facebook intervention groups, in which peer leaders moderated discussions about HIV testing and infection prevention, were about three times more likely to request a home-based HIV testing kit than those in Facebook groups that received only general health information (*Am J Public Health*, 104:1707-12, 2014). Upon reading of their success, researchers in Peru contacted Young to implement a Facebook-based intervention program there, and got very similar results, with participants three times more likely to get tested for HIV. (*Lancet HIV*, 2:e27-32, 2015). Young and his colleagues are now gearing up to test this social media–based intervention strategy in a five-year randomized controlled trial.

So has social media helped or hurt the HIV community? According to Simon Rosser, director of the HIV/STI Intervention and Prevention Studies (HIPS) Program at the University of Minnesota School of Public Health, that's the wrong question to ask. "Something as complex as new technology and media, it's like asking the question, 'Is the Internet good or bad?' It's just too simplistic a question when framed that way," says Rosser. On the other hand, "asking what are the effects of the Internet, how is it changing society, and what are the benefits and risks inherent in it is very, very helpful, even critical." —Jef Akst

## Miraculous Activist

In 2006, Timothy Ray Brown was bicycling through Berlin on his lunch break. He regularly cruised the city on two wheels, making a daily 14-mile round trip for work. But on this day, he got off and walked, too tired to make it back to work. "I called my partner and said, 'I need to get an appointment with a doctor,'" Brown recalls.

Blood tests led to a bone marrow biopsy, which led to a diagnosis of acute myeloid leukemia. From there a remarkable series of circumstances—some fortuitous, others debilitating—catapulted Brown into medical history: to this day he remains the only person who has been cured of AIDS.

Cure is not a word the HIV/AIDS community throws around lightly. "Nobody would dare to use the word 'cure' before this happened," says James Hoxie, an HIV researcher at the University of Pennsylvania. But Brown's cure "has generated an entirely new field of science that we boldly call cure or eradication research. It's n=1, but it transformed our way of thinking about AIDS." It has also profoundly impacted Brown, and not just in terms of his health. His remarkable medical history has turned the unassuming translator from Washington State into a jet-setting celebrity whose very existence, as Hoxie says, gives people hope.

**THE BERLIN ACTIVIST:** Timothy Ray Brown has gone from medical marvel to HIV/AIDS activist, spreading the hope that researchers can eventually develop a cure for the disease.

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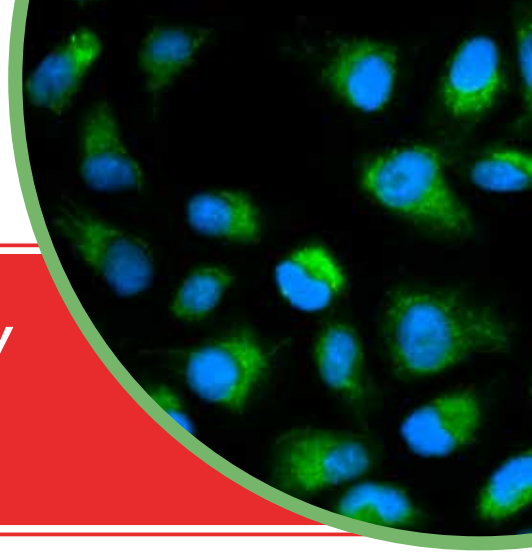

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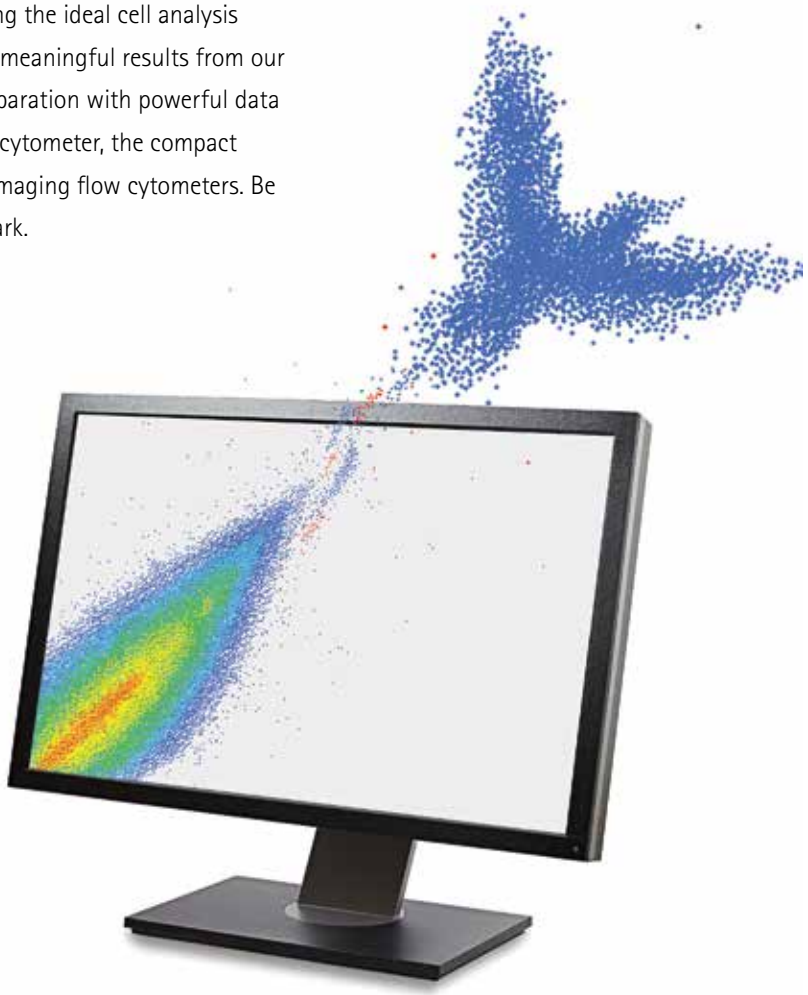

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**We need to find better ways and less expensive ways and less dangerous ways of curing AIDS.**

—Timothy Ray Brown, the Berlin patient

The oncologist that Brown contacted for treatment in 2006 was Gero Hütter. When Hütter learned his patient was HIV-positive, he recalled his firsthand experience with AIDS 10 years earlier as a medical student in the pre-antiretroviral therapy era. "I saw patients with AIDS dying, and some were younger than me," says Hütter. He also remembered reading a study around that same time about some people's ability to naturally ward off the viral infection. That paper left him with the impression that perhaps these people held the key to stopping HIV.

About one percent of people with European heritage have a natural immunity to HIV that stems from a homozygous mutation (called delta32) in the gene for the CCR5 coreceptor on T cells. The mutation cripples the receptor's function, thereby blocking HIV from using CCR5 to enter cells. Brown was in need of a bone marrow transplant to treat his leukemia, and Hütter had the wild idea that perhaps he could find a donor with the delta32 mutation whose stem cells could replace Brown's immune system—and treat not only his malignancy, but maybe his HIV infection as well.

Sixty donor matches, tested one by one, were negative for a homozygous mutant, but the 61st came back positive. In 2007, Brown received the transplant and stopped taking his antiretroviral medication. He spent the next two weeks in isolation at the hospital before returning home. Eventually, he went back to work, started exercising again, and even gained some weight. Three months after his transplant, there was no detectable virus in his system.

Brown experienced setbacks: pneumonia, a relapse of leukemia, a second bone marrow transplant, neurological problems. Still, the HIV was kept at bay. He gained notoriety in the medical and lay press as the "Berlin patient," opting to remain anonymous. His case gave researchers and patients the idea that a cure was possible. But for years Brown stayed out of the limelight. "I basically was in recovery still," he says. At the end of 2010 he decided to speak with the media and reveal his identity. "I didn't want to be alone in my club.

Brown revealed his identity first to the German magazine *Stern*, then to others. His face, his name, and his story gave hope to the HIV/AIDS community. He symbolized the idea of a cure, Brown says. He traveled internationally to speak and attend meetings. But as he realized the significance of his experience, Brown wanted to speak on behalf of his own organization, rather than others'.

In June 2012, Brown met AIDS activist Dave Purdy, and the two joined forces to launch the Cure for AIDS coalition, dedicated to disseminating information on cure research. "The focus is on making sure we support the entire cure community . . . using Timothy and his celebrity to get the word out," says Purdy. Last year, the team put out the first Cure AIDS Report, an extensive collection of articles and resources available on the coalition's website. The report also aims to connect patients with clinical trials and researchers with funding.

Another project of the coalition is to develop a registry of people who are homozygous for the *CCR5* delta32 mutation. Not only would clinicians and researchers have access to more data and material, but volunteers who have the mutation and want to contribute to cure research would have an opportunity to participate, says Purdy. Hoxie, who is a homozygote himself, has given blood numerous times, and even donated stem cells. (See "Scientist as Subject," *The Scientist,* October 2010.) He says if asked he'd be happy to donate to the registry.

Although the experience with his bone marrow transplants relieved Brown of a chronic infection and gave him a new avocation, he says he wouldn't wish the experience on his worst enemy. Chemotherapy, radiation, bone marrow transplants, and brain surgery have all left his body wrecked. "We need to find better ways and less expensive ways and less dangerous ways" of curing AIDS, he says.

—Kerry Grens

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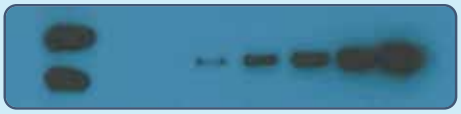

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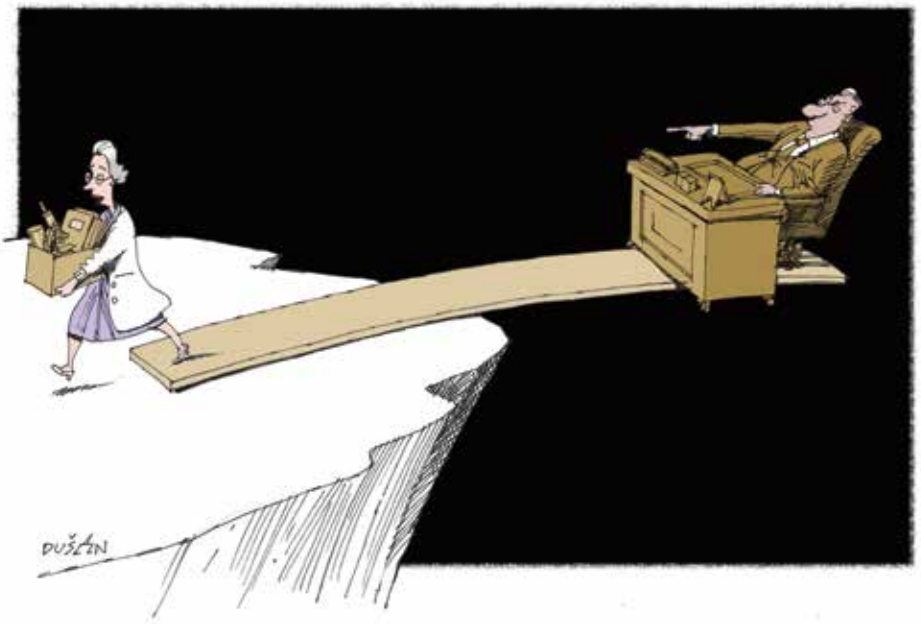
# Think Before You Fire

Industry layoffs may save a few dollars, at the cost of losing the collective brainpower of thousands of scientists.

BY SARAH RAMSAY

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Have you ever been laid off? It happened to me for the first time this February, along with a considerable number of my colleagues, two years after my longtime employer—a truly innovative and forward-thinking midsize pharmaceutical company—was purchased by a larger pharma company. As is customary in such situations, a security guard escorted me to my office, where I was instructed to pack my things into two cardboard boxes. It was an altogether surreal experience, one that some of you may know all too well. Layoffs have become an unfortunately familiar part of life in the pharmaceutical industry, and R&D is always low-hanging fruit in such situations, as we do not make money for the company in the short term. Scientists continue to be let go at an alarming rate, and are often unable to find work no matter their level of experience.

The evening of my layoff I found myself sitting on the roof of my car repairing the light in my garage door opener. Of course, it had chosen that day to malfunction. As I was soldering in the door opener's new logic board, I started thinking about all of the other laid-off scientists and about the waste of collective brainpower. Even those who haven't been let go, or who are able to find new jobs, are feeling more and more restricted and limited in their research, constantly oppressed by the pressure to do more with less and in less time. It seems that industry has forgotten that science takes time, and when you push it, bad things often happen.

And so I decided to explore the ramifications of layoffs both on a personal and societal level. I talked with several of my scientist friends and former colleagues, including academics and those working in industry, from bench scientists all the way up to senior VPs. I also spoke with current and future graduate students and to people tangentially associated with R&D, including project managers and quality-control professionals. What I found was a creativity and productivity drain—an enormous underutilization of brainpower that could and should be answering the most challenging questions facing the human race and the planet.

**Scientists who are lucky enough to be employed are finding their creativity and ingenuity stunted by shrinking R&D budgets and a lack of autonomy.**

A scientist out of work is like a fire with nothing to burn. Problem solving and exploration are the fuel that keeps our fires burning—hence my garage-door-opener–fixing session the day I was laid off. Having something to fix and puzzle over kept my mind occupied and got me through a difficult time far more effectively than any movie or television show could have. I found this a common theme in the conversations I had with other laid-off researchers over the next few weeks. One of the scientists I spoke with has been looking for work for more than a year and talked of how he missed the intellectual stimulation even more than the paycheck.

I also found that those scientists who are lucky enough to be employed are finding their creativity and ingenuity stunted by shrinking R&D budgets and a lack of autonomy. They spoke of how rarely they were asked for their opinions and how increasingly common it was to have decisions supposedly based on science made by nonscientists. They also spoke of the ever-pres-

ent worry about being laid off and what that would mean not just for them, but for their research projects. Typically, there is no debriefing when a researcher is laid off, and much of the "soft" information gathered over the life of the project—such as ideas jotted down on scrap paper or knowledge stored away in the scientist's head—is lost.

For some, the unsteady pharmaceutical research environment became too much to bear. One senior-level scientist with decades of experience decided to leave science altogether after suffering the consequences of repeated mergers. Others were driven back to academia, such as the former president of a major biotech company who decided to leave his position and return to the bench at a local university, realizing that his brain needed an outlet that he could not find in the confines of upper management. But even academia is no safe haven from economic pressures. When I spoke to academic researchers, I heard less about autonomy and job security than about the never-ending grant application process, the dismal pay for bench scientists and postdocs, and the increasing level of bureaucracy pulling scientists away from research and into meetings that appear to accomplish very little.

I also talked with present and future graduate students, who spoke of being lured away by parents and teachers into professions with greater security and higher pay. After all, there are far more lucrative and easier fields than science for these young men and women to enter. It is imperative that, instead of redirecting these incredible young minds, we nurture their talent and enthusiasm for scientific research. If we do not, we risk losing an entire generation of potential.

But there is hope found in research centers around the world that are transforming how we conduct science. Take, for example, the not-for-profit Cell Therapy Catapult in London, established in 2012 as a center of excellence in innovation. Its mission is to "drive the growth of the industry by helping cell therapy organizations across the world translate early-stage research into commercially viable and investable therapies." It bridges the often difficult divide between academic institutions, for-profit businesses, and other research communities. In other words, it allows scientists to be their most creative and productive while in turn advancing the business of cell therapy. This model, and models similar to it, could and should be utilized in other research disciplines. Collective brainpower is an invaluable commodity.

**Focusing solely on the bottom line while not taking into careful consideration the impact that R&D cutbacks and layoffs will have on the scientific, technological, and therapeutic advances of the future will cost us far more than money.**

We all know that drug development, clinical trials, and other aspects of biomedical research are extremely expensive. Yet focusing solely on the bottom line while not taking into careful consideration the impact that R&D cutbacks and layoffs will have on the scientific, technological, and therapeutic advances of the future will cost us far more than money. We will lose generations of scientists to other careers, and with them the breakthroughs, cures, and pure genius that will shape our collective tomorrows. ■

*Sarah Ramsay is a biochemist and cell biologist living in Fort Worth, Texas. She has spent the last 11 years working in the field of regenerative medicine with an emphasis on wound healing.*

# Seeded by Weeds

More than 50 years after cross-contamination of cultured cell lines was recognized, the problem continues to plague the scientific community.

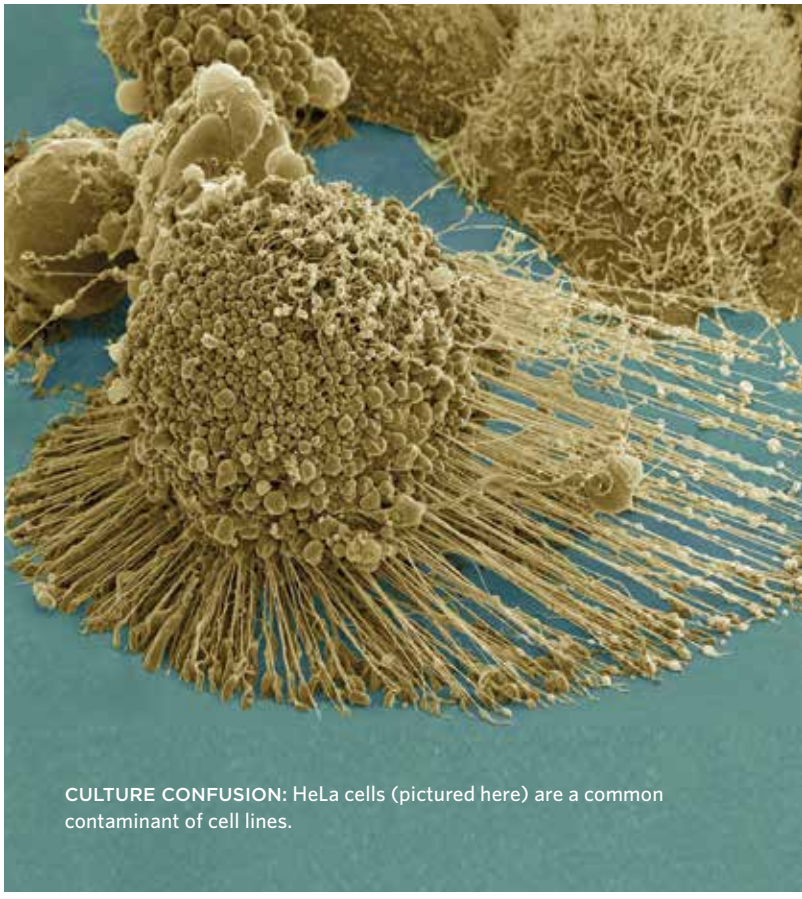
BY K. JOHN MORROW JR.

In the early days of cell culture, researchers often struggled to get their cell lines to survive for long periods of time. At first, some investigators blamed inappropriate culture conditions and kept tweaking the recipe, hoping to get it right. HeLa cells, first cultured in 1951 from cervical cancer tissue, were cloned in 1955 and became the first human cell line capable of permanent growth in culture. Thereafter, dozens of other permanent human cell lines were reported in the literature, suggesting that they were relatively easy to establish. However, in the 1960s, Leonard Hayflick and his colleagues published a landmark series of papers explaining the initial difficulties in getting cells to grow indefinitely: normal human fibroblasts grown in culture divide a finite number of times.

Around this time several reports suggested that a number of permanent cell lines were not what their authors claimed. Then, at a National Cancer Institute meeting in 1966, Stanley Gartler of the University of Washington in Seattle presented evidence that a large number of long-lived cell lines believed to be of independent origin were in fact contaminated by HeLa cells—which, likely related to their cancerous nature, were indeed immortal (*NCI Monograph*, 26:167-95, 1967).

Hayflick's results regarding a cell's doubling limit in culture should have immediately raised concern about reportedly long-lived human cell lines. Nevertheless, Gartler's findings were met with shock from the cell-culture community. "Everybody knew that HeLa was robust, but who was going to admit that their lab had messed up?" Gartler told me in a recent discussion. "Besides, these people [wanted to believe] that they had produced another HeLa."

Since then, instances of culture contamination with HeLa and other cell types have been reported over and over again, decade after decade. Perhaps the best demonstration of the overwhelming ubiquity of HeLa contamination appeared just earlier this year, when Paul Cantalupo of the University of Pittsburgh and his coworkers described how HeLa sequences have muscled their way into the Cancer Genome Atlas (*J Virol*, 89:4051-57, 2015). Specifically, the authors identified sequences of human papillomavirus (HPV), a causative agent of cervical cancer, in numerous non-cervical cancer genomes, as well as in the genomes of some normal tissues. The sequences included the junction between the HPV genome and the HeLa genome, suggesting that those cell lines weren't themselves infected with the virus, but rather contaminated with HeLa, which does contain integrated HPV sequences.



**CULTURE CONFUSION:** HeLa cells (pictured here) are a common contaminant of cell lines.

NATIONAL INSTITUTES OF HEALTH/WIKIPEDIA

**Contamination events may be responsible for widespread irreproducibility of preclinical studies.**

At the same time, researchers have continued to publish studies using misidentified cell lines. Most disturbing are contamination events that go unreported, but may be responsible for widespread irreproducibility of preclinical studies. Pharma companies are constantly surveying the literature for reports that describe promising drug candidates, with the goal of moving such compounds through the R&D pipeline. However, such leads often fail to pan out, as subsequent experiments

fail to replicate the results of the original study. In one series of investigations, researchers at the biopharmaceutical firm Amgen were only able to duplicate 6 of 53 peer-reviewed academic studies of potential drug candidates (*Nature*, 483:531-33, 2012). "The basic problem remains that non-geneticists simply don't think in terms of identifying their material genetically," Gartler says.

Jon Lorsch of the National Institute of General Medical Sciences and colleagues have argued that the scientific community needs to work together to ensure standards of integrity and reproducibility for authenticating cell lines (*Science*, 346:1452-53, 2014). One change that has already been implemented is the mandatory screening of cell lines imposed by some journals and granting agencies. At least 22 journals now require that authors testify to the authenticity of their cell lines, but it is too soon to say whether this policy change will impact the poor reproducibility that plagues translational research.

In the meantime, Bob Geraghty of the Cancer Research UK Cambridge Institute at the University of Cambridge and colleagues have published an extensive collection of guidelines for proper handling of cells in culture (*Br J Cancer*, 111:1021-46, 2014). Topics including authentication and characterization of cell lines are dealt with at length, and are aimed at "those who may, despite years of experience, have allowed suboptimal procedures to become part of local practice." They recommend cell line authentication using DNA-based methods, specifically, short tandem repeat (STR) profiling, which identifies sequences of DNA that are highly variable between individuals.

Nearly five decades after rampant cross-contamination of cell lines was clearly demonstrated by Gartler and others, it remains a significant—and costly—problem. University of Colorado geneticist Christopher Korch has estimated that the costs of using mislabeled cells may be as high as $2.8 billion for just two lines, HEp-2 and INT 407. Korch arrived at this figure by estimating all the costs that go into a scientific paper, and multiplying by the number of papers that used these cell lines inappropriately. Because many of the studies were investigations of broad scientific questions and did not require a specific cell line, the actual loss to society may be less, although it is clearly substantial.

Reports in the literature that cannot be substantiated, especially in the field of cancer research, can cause great loss of time and resources, and may result in the fruitless pursuit of unworkable drug candidates. The fact that this corrosive problem has persisted for so long raises pessimism that it will never be fully eliminated. At a time when the credibility and integrity of the scientific enterprise is questioned by the public, the stakes could not be higher. ■

*K. John Morrow Jr. is president of Newport Biotechnology Consultants, which performs consulting and writing services for the biotechnology industry.*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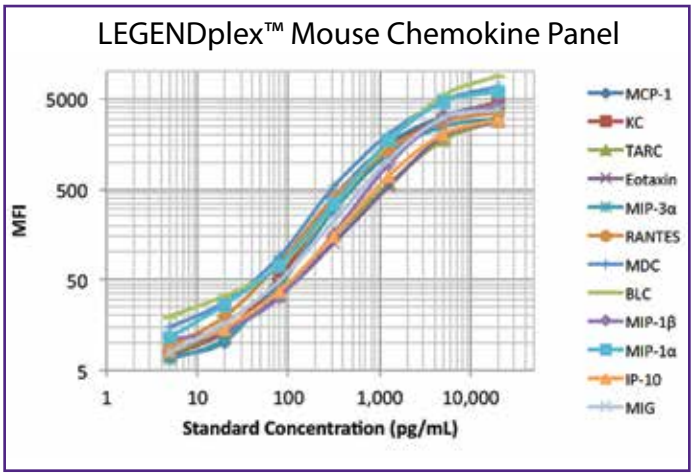


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# Scanning for SIV's Sanctuaries

Whole-body immunoPET scans of SIV-infected macaques reveal where the replicating virus hides.

BY RUTH WILLIAMS

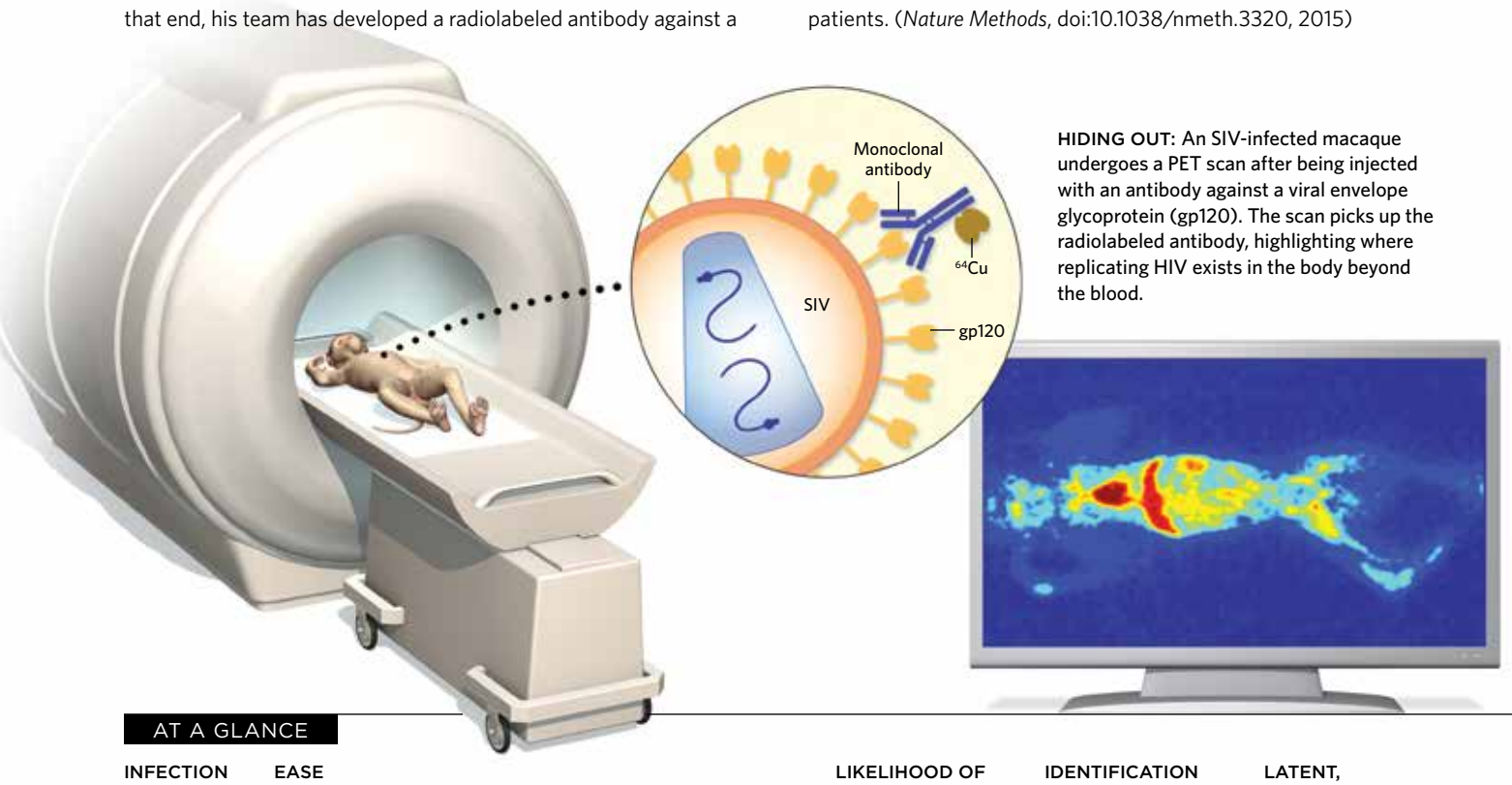
Antiretroviral drug treatment of HIV-positive patients reduces the virus to an undetectable level, but in reality it's not eradicated. The virus still lurks in secluded locations around the body. "If you stop giving the drugs, the virus comes back," says Tom Hope of Northwestern University's Feinberg School of Medicine in Chicago.

The problem, Hope says, is that "almost everything we know about this disease comes from studying the blood, and that does not reflect what's going on in the different tissues." The only option for studying body tissues has been to perform biopsies, which are invasive and often don't provide a complete picture of the whole tissue, let alone the extent of the body.

François Villinger of Emory University in Atlanta realized there "needed to be a way to quantify virus throughout the entire body." To that end, his team has developed a radiolabeled antibody against a viral envelope glycoprotein in the nonhuman primate version of HIV—SIV—and used it to track replicating virus in infected macaques.

Positron emission tomography (PET) scans detected antibody signals in locations expected to be SIV hot spots—the gut and reproductive tract—and some locations that were less expected: the lungs and nasal cavity. Crucially, while antiretroviral treatment reduced the PET signal from these sites, it was not always eradicated. Similar sites of persistent, minimal viral replication were also apparent in untreated animals that had naturally suppressed infection—so-called elite controllers.

Determining precisely where these pockets of surviving viruses are located "will be invaluable information" for designing new drugs, says Hope. Moreover, the PET technique will enable the efficacy of such drugs to be readily assessed in macaques and, ultimately, in patients. (*Nature Methods*, doi:10.1038/nmeth.3320, 2015)




**HIDING OUT:** An SIV-infected macaque undergoes a PET scan after being injected with an antibody against a viral envelope glycoprotein (gp120). The scan picks up the radiolabeled antibody, highlighting where replicating HIV exists in the body beyond the blood.

## AT A GLANCE

| INFECTION TRACKING TECHNIQUE | EASE OF METHOD | LIKELIHOOD OF DETECTING VIRAL RESERVOIRS | IDENTIFICATION OF INFECTED CELL LINEAGE | LATENT, NONREPLICATING VIRUS DETECTED? |
|---|---|---|---|---|
| Biopsy | Depends on tissue; presence of virus determined by PCR or immunohistochemistry. | Hit-or-miss | Yes | Yes, by PCR |
| ImmunoPET | Fairly easy; subject injected with radiolabeled antibody, then scanned. | Good | No | No |

# Hidden Menace

Curing HIV means finding and eradicating viruses still lurking in the shadows.

BY GENEVIEVE MARTIN, MATTHEW PACE, AND JOHN FRATER

Since the early 1980s, when HIV was first identified, our knowledge of the virus—how it causes disease, how it interacts with our immune system, how it responds to drugs—has grown year by year. Drugs specifically designed to target HIV, and given as a cocktail of different agents—known as combination antiretroviral therapy (ART)—have decreased the mortality associated with HIV infection to the point where, for newly diagnosed individuals today, life expectancies are comparable to those who are HIV-negative.

But of the 35 million people currently living with HIV, the World Health Organization estimates that only around 40 percent use ART, partly because about half do not know they are infected. Providing ART to all who need it is a major challenge, and even when the drugs are available they are not a panacea. Regardless of treatment, there is increasing evidence that HIV-infected individuals may be at greater risk of non-AIDS comorbidities, for example, cardiovascular disease and dementia. Moreover, ART has to be taken for life: if the drugs are stopped, virus production quickly ramps up and the disease can progress, a phenomenon known as rebound.

Rebound occurs because HIV forms a reservoir in long-lived T cells that persists despite treatment. As with all retroviruses, a key aspect of the HIV replication cycle is the reverse transcription of the viral genome into DNA, followed by integration of this viral DNA, known as the provirus, into the host genome. (See illustration on page 36.) In activated cells, this proviral DNA can give rise to viral mRNA, proteins, and infectious viral particles. However, in some infected cells, the virus enters a resting state, termed latent infection, in which transcription or translation is restricted but integrated HIV is still present. These latently infected cells make up the HIV reservoir and, eventually, may be stimulated to produce infectious virus.

**One challenge in targeting latently infected cells is that they do not produce HIV antigens and are therefore indistinguishable from uninfected cells.**

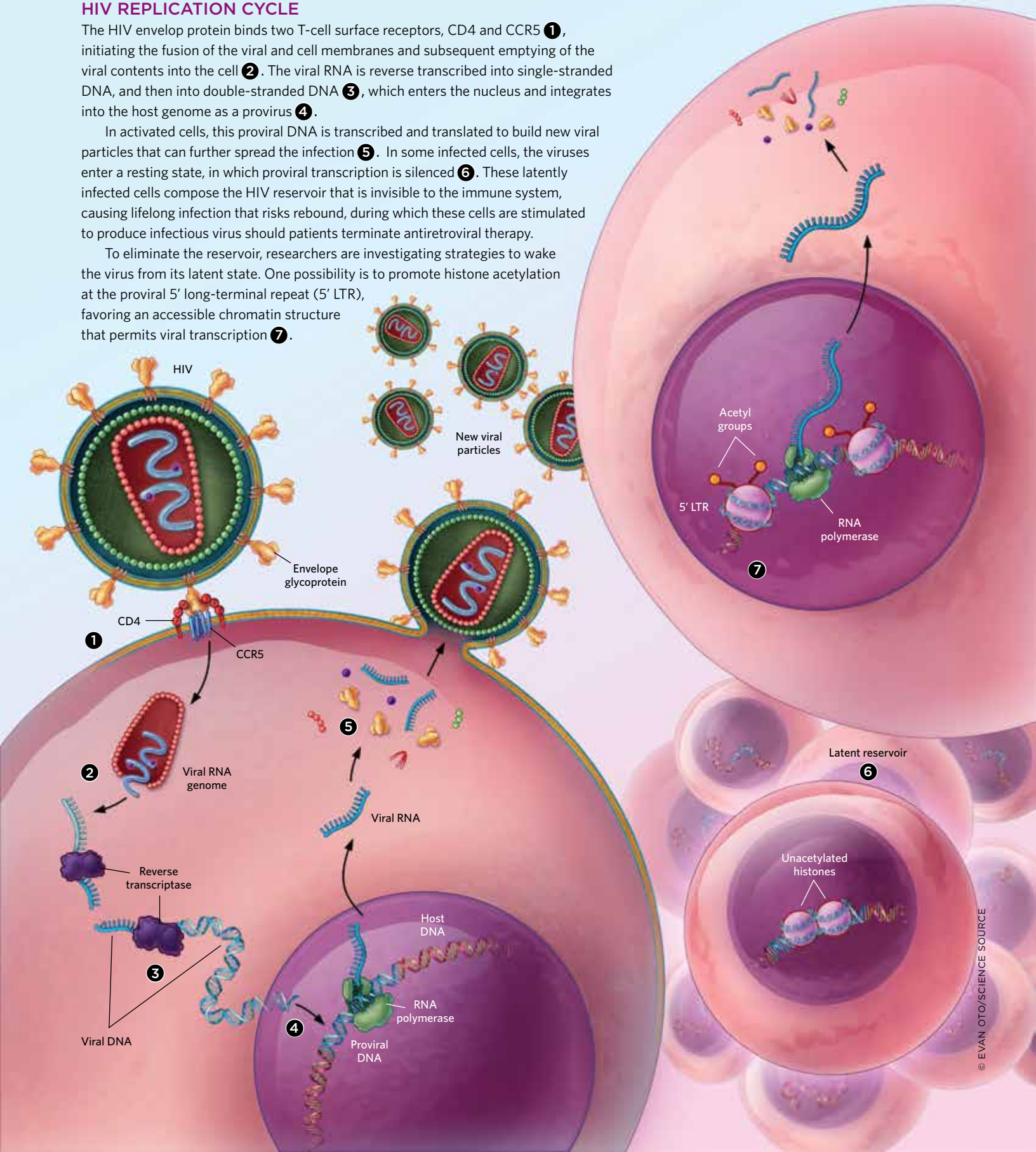
The HIV reservoir consists largely of resting $CD4^+$ T cells, but other cells, such as macrophages, may also contribute. In patients who have been treated for many years with ART, these latently infected cells are rare, but still present. It has been estimated that the proportion of latently infected cells capable of giving rise to rebound virus production is approximately one in a million resting $CD4^+$ T cells in patients on ART.[1] However, the difficulty of reliably measuring the reservoir means this number could be significantly higher or lower. (See “Ethical Dilemmas” on page 38.) Regardless, the HIV reservoir is a major barrier to virus eradication, and its existence raises several questions for cure

## HIV REPLICATION CYCLE

The HIV envelop protein binds two T-cell surface receptors, CD4 and CCR5 ❶, initiating the fusion of the viral and cell membranes and subsequent emptying of the viral contents into the cell ❷. The viral RNA is reverse transcribed into single-stranded DNA, and then into double-stranded DNA ❸, which enters the nucleus and integrates into the host genome as a provirus ❹.

In activated cells, this proviral DNA is transcribed and translated to build new viral particles that can further spread the infection ❺. In some infected cells, the viruses enter a resting state, in which proviral transcription is silenced ❻. These latently infected cells compose the HIV reservoir that is invisible to the immune system, causing lifelong infection that risks rebound, during which these cells are stimulated to produce infectious virus should patients terminate antiretroviral therapy.

To eliminate the reservoir, researchers are investigating strategies to wake the virus from its latent state. One possibility is to promote histone acetylation at the proviral 5′ long-terminal repeat (5′ LTR), favoring an accessible chromatin structure that permits viral transcription ❼.



strategies. Is it possible to completely eradicate latently infected cells from the body, or can we keep them silent to prevent viral rebound? Even more to the point, is it possible to prevent the reservoir from forming in the first place?

## Different types of cure

Due to the assimilation of viral genetic elements into the host genome, researchers previously assumed that, once infection has taken hold, HIV can never be completely eliminated from the body. Yet in 2009, German clinicians announced the case of an apparent HIV cure in Timothy Ray Brown, also known as "the Berlin patient."[2] Brown underwent a bone marrow transplant following unsuccessful treatment for acute myeloid leukemia with conventional chemotherapy. The bone marrow donor selected by Brown's clinicians was homozygous for a mutation in the CCR5 gene, preventing the expression of the CCR5 HIV coreceptor on the surface of T cells and conferring a high degree of natural resistance to HIV infection. Following the transplant, Brown ceased taking ART, and the virus did not rebound. More than six years later, researchers have been unable to find evidence of replication-competent HIV in blood or tissues from this patient; it appears that any viral reservoir has been cleared.[3] Despite the exceptional circumstances surrounding this case, many believe that the Berlin patient serves as proof of concept that HIV can be cured.

Researchers have since attempted bone marrow transplants from donors carrying the same *CCR5* mutation in six other cases of HIV-positive patients. Unfortunately, all of these individuals died within a year from relapsed malignancy or transplantation complications.[4] In one of these individuals, rebound occurred after an HIV variant used an alternative T-cell coreceptor, CXCR4, suggesting a potential limitation to targeting only CCR5.[5]

These cases demonstrate the intrinsic dangers and difficulties of the Berlin patient strategy, which could never be realistically scaled up to help all those infected with HIV. Interestingly, when two patients in Boston underwent bone marrow transplants using tissue from a donor carrying wild-type *CCR5*, their viral levels dropped to undetectable levels, both in plasma and intracellularly, and these low levels endured for several years.[6] Unfortunately, viral rebound occurred within a few months of stopping ART, indicating that the *CCR5* mutation was indeed critical to the Berlin patient's cure.[7] This has led researchers to another strategy to eradicate HIV: using gene therapy to turn off *CCR5* expression. If successful, such a treatment could prevent additional cells from being infected with HIV, thwarting disease progression even in the presence of a viral reservoir. (See "Genome Editing Cuts Out HIV," *The Scientist*, July 21, 2014.)

In addition to preventing the spread of the virus to new cells within body, efforts to "cure" HIV have focused on reducing the size of the reservoir to achieve a remission or functional cure, in which patients could remain off therapy without rebounding, despite detectable HIV DNA in their bodies.

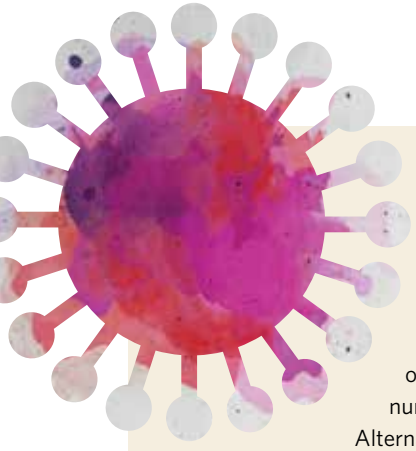
## Early treatment, better control

While researchers have traditionally envisaged a "sterilizing cure," in which the virus is completely eliminated from the body, this may not be necessary for controlling the infection. In addition to preventing the spread of the virus to new cells within body, efforts to "cure" HIV have focused on reducing the size of the reservoir to achieve a remission or functional cure, in which patients could remain off therapy without rebounding, despite detectable HIV DNA in their bodies.

Serving as an example of functional cure, 14 patients across France known as the VISCONTI cohort have successfully stopped ART without return of virus production, some for many years. Unlike the Berlin patient, these patients still have detectable, although small, HIV reservoirs that could serve as a future source of viral reactivation.[8] Nevertheless, the fact that these patients have achieved long-term posttreatment control (PTC) could provide important clues for the development of an HIV cure.

A key factor with the VISCONTI cohort is that these individuals started treatment very soon after being infected. Although not all patients treated early are able to cease ART without rebound, several other cohorts that commenced ART during early stages of infection have also achieved varying levels of PTC. While viral rebound occurs rapidly in most individuals, between 5 percent and 15 percent of patients remain free of virus at 24 months after ART cessation.[8,9] In contrast, PTC is rarely, if ever, observed among patients who commenced ART only after their $CD4^+$ T cell counts declined below a cut-off point, suggesting that starting treatment during primary HIV infection appears to be important for controlling the virus. Further evidence for the potential of this strategy comes from the reported cure of an infant in Mississippi who, perinatally infected with HIV, commenced ART 30 hours after birth.[10] Treatment was discontinued at 18 months of life, and HIV levels remained undetectable for more than a year, until rebound occurred in July 2014.[11]

The SPARTAC (Short Pulse Antiretroviral Therapy at HIV Seroconversion) trial put early ART to the test in a large randomized trial that ran from 2003 to 2010 across eight countries. Adults testing positive for HIV received a short course of ART within 24 weeks of seroconversion, when an HIV-specific antibody becomes detectable in the blood, resulting in delayed $CD4^+$ T cell decline. The trial also showed that levels of HIV DNA correlated with this delay and predicted time to viral rebound, a finding that links early treatment with reduced reservoir size, possibly as a result of lim-

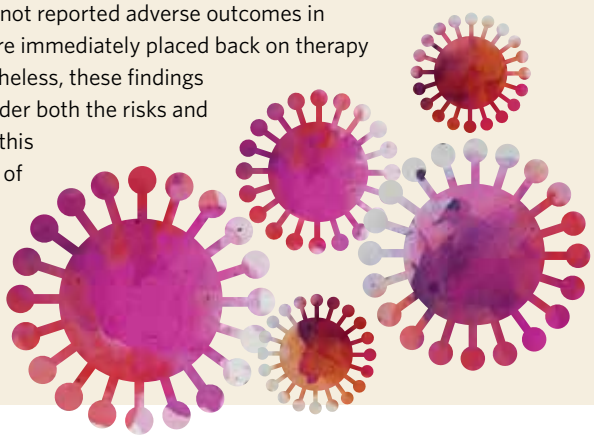


## ETHICAL DILEMMAS

One problem researchers face in evaluating HIV cure strategies is measuring the size of the latent reservoir. Several assays exist to quantify the reservoir, each with significant limitations; it is likely that these assays either over- or underestimate its size. Estimates of the number of copies of proviral DNA, for example, include a large number of integrated viruses that appear to be nonfunctional. Alternatively, the assay used to measure only replication-competent copies of the virus may underestimate the true number because it cannot activate all such copies.

In the absence of robust methods for detecting latently infected cells, treatment interruption remains the sole way of testing HIV cure interventions. Ceasing therapy, however, risks resumption of HIV production and reseeding of the reservoir, which may impact disease progression and allows for transmission of the virus.

Evidence of adverse outcomes after treatment interruption comes from the SMART study, in which such interruption was associated with increased mortality, opportunistic infections, and major non-AIDS comorbidities in chronically infected patients (*N Engl J Med*, 355:2283-96, 2006). Importantly, the SMART study left patients untreated for long periods of time despite high levels of viremia. Researchers have not reported adverse outcomes in studies where patients were immediately placed back on therapy after viral rebound. Nevertheless, these findings highlight the need to consider both the risks and the ethical implications of this strategy. Close monitoring of patients during treatment interruption with clear plans for restarting ART need to be part of cure intervention trials.



iting the initial seeding of the reservoir.[12,13] Because it is almost certain that a cure will be easier to achieve in patients with lower numbers of latently infected cells, it seems that early treatment will be an important part of HIV eradication strategies.

### Reversing latency

As most patients are not diagnosed with acute HIV infection and are unable to initiate ART early on, treatments are also needed to deplete the HIV reservoir once it is established. One challenge in targeting latently infected cells is that they do not produce HIV antigens and are therefore indistinguishable from uninfected cells. This transcriptional silence renders these cells invisible to immune surveillance and allows persistence of provirus over time. Although counterintuitive, waking the virus from its latent state may be the key to eradicating the reservoir, by rendering reservoir cells susceptible to immune clearance and other targeted treatments. Researchers are developing strategies combining drugs to reactivate latent virus and techniques to boost immune clearance of infected cells—a so-called kick-and-kill approach.

Transcription of proviral HIV DNA is dependent on the recruitment of appropriate transcription factors to the viral 5' long-terminal repeat (5' LTR). As with all genes, chromatin arrangement around the site of viral integration is an important regulator of transcriptional status. High histone acetylation at the 5' LTR is associated with an accessible chromatin structure, favoring transcription. Acetylation status is maintained by a balance between histone acetyltransferases, which act to promote acetylation, and histone deacetylases (HDACs), which decrease acetylation. HDAC inhibitors are drugs that promote nonspecific acetylation and activate cells latently infected with HIV both in vitro and in vivo.

Vorinostat is an HDAC inhibitor used to treat cutaneous T-cell lymphoma in humans. It has been shown to disrupt HIV latency in cellular models and in primary $CD4^+$ T cells from HIV-infected patients. Trial doses of vorinostat given to HIV-infected individuals with ART-suppressed viral replication caused increases in cellular acetylation and subsequent HIV transcription, but with no evidence of rebound.[14,15] In other words, the latent cell was activated enough to start the virus life cycle, but no new virions were detected. This could be due to posttranscriptional barriers to viral expression, such as mRNA degradation, or suboptimal dosing regimens. Although it is unclear what level of expression is needed to trigger immune clearance, it is presumed that at least protein expression, if not assembled virus, will be needed. Researchers at Aarhus University in Denmark have shown that two other HDAC inhibitors, panobinostat and romidepsin, have higher potency and can induce virus production in patients treated with ART.[16,17] Despite reactivation of latent viruses, however, neither of these drugs resulted in decreases in reservoir size. But given the short time period of these studies and the small number of patients, this is not altogether unexpected, and the results serve as evidence that it is possible to reactivate the reservoir in vivo.

Although HDAC inhibitors are the most widely studied class of HIV-activating drugs, several other candidates may also be able to reverse HIV latency. These include methyltransferase inhibitors, protein kinase C agonists (prostratin and bryostatin), the BET bromodomain-inhibiting molecule JQ1, and the zinc-chelat-

ing agent disulfiram. The distinct mechanisms of these agents mean that toxicities and efficacy will differ, and it is unclear which agents will be most suited for clinical use. Human trials using HDAC inhibitors and these other HIV-activating agents are exploring the effect of these drugs on viral transcription and translation, as well as the drugs' safety and tolerability.

Even if HIV-activating agents can provide the kick needed to disrupt HIV latency, there is no evidence that this will result in clearance of the reservoir by the immune system alone. As such, it is likely that these drugs will need to be used in combination with other strategies to promote immune clearance of these cells. The RIVER (Research in Viral Eradication of HIV Reservoirs) trial, for example, is combining vorinostat with vaccination in patients on ART in the U.K. A similar study in Denmark using the HDAC inhibitor romidepsin, and a different vaccine candidate is also underway.

## Putting cure into context

Significant work remains to be done in the development of a potential cure for HIV. It is an exciting time in the field, with interventional trials running in parallel with continued basic research into the mechanisms of HIV infection and pathology. In addition, a number of large observational studies of the dynamics of reservoir size are underway to understand PTC, which may provide insights into factors that predict its occurrence, the mechanisms of reservoir formation and maintenance, and the probability of rebound.

Meanwhile, several different therapeutic approaches to an HIV cure are currently under investigation. In addition to gene therapy attempting to damage the CCR5 gene or block its expression, researchers are developing therapeutic HIV vaccines. (See "Defeating the Virus" on page 40.) Antibodies with broad HIV-neutralizing capability may have some in vivo activity against the virus, and transfusions of these are being trialled. Coupled with HIV-activating drugs, these immune augmentation strategies are important early steps towards a cure.

Of course, research into an HIV cure is only one of a multitude of approaches to solving the challenges posed by this virus. Prevention, testing, treatment, and managing comorbidities remain the cornerstone of HIV management and research worldwide. Curative interventions, while exciting, are still in very early stages of development; it is unlikely that these are going to play an important role in treatment of infected patients in the near future. Despite this, the prospect of an HIV cure remains a driving force for many, and a successful cure must, in some way, address the reservoir. In this rapidly progressing field, the next few years will be crucial in determining whether clearance of the reservoir may one day be possible.■

**Although counterintuitive, waking the virus from its latent state may be the key to eradicating the reservoir, by rendering reservoir cells susceptible to immune clearance and other targeted treatments.**

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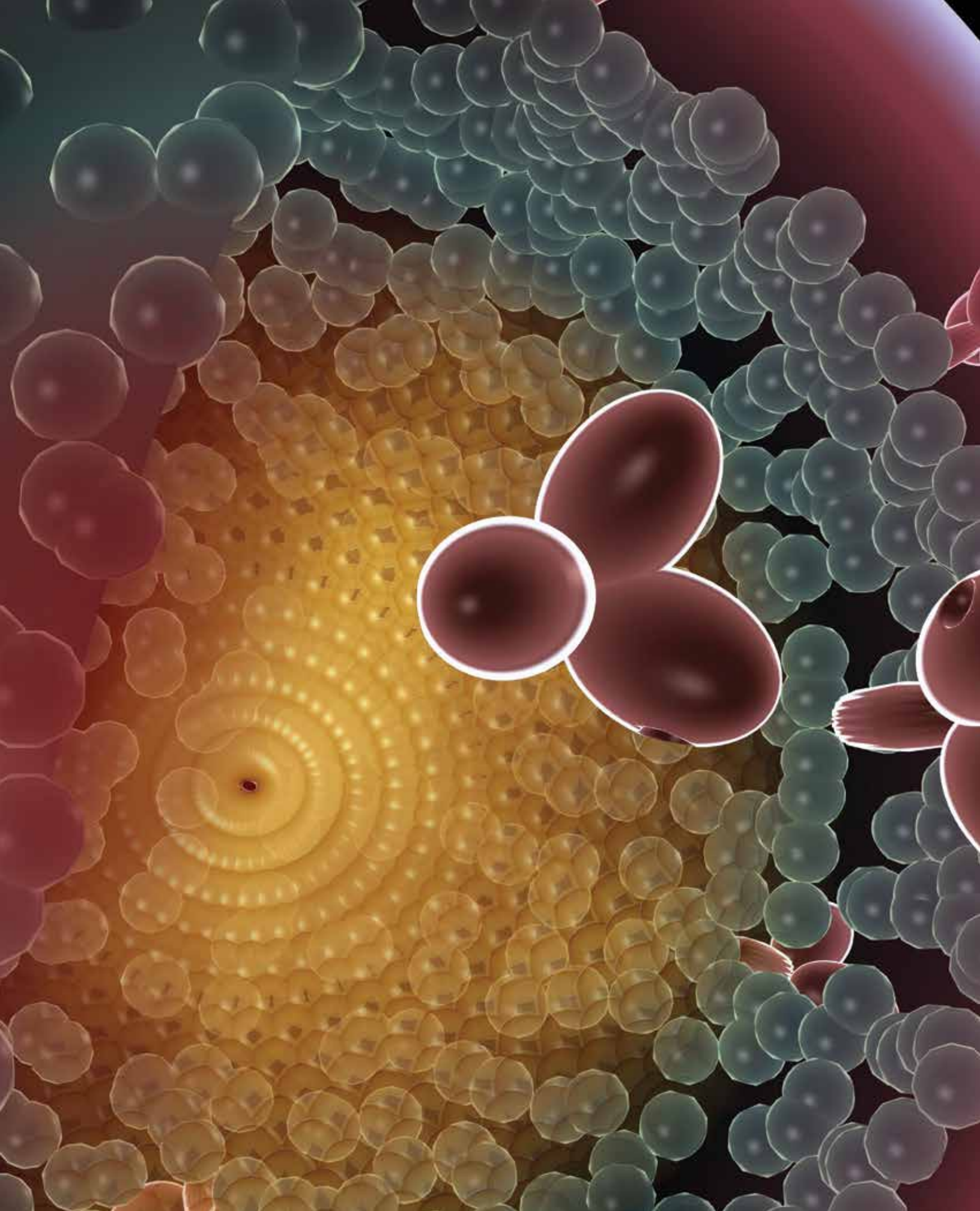
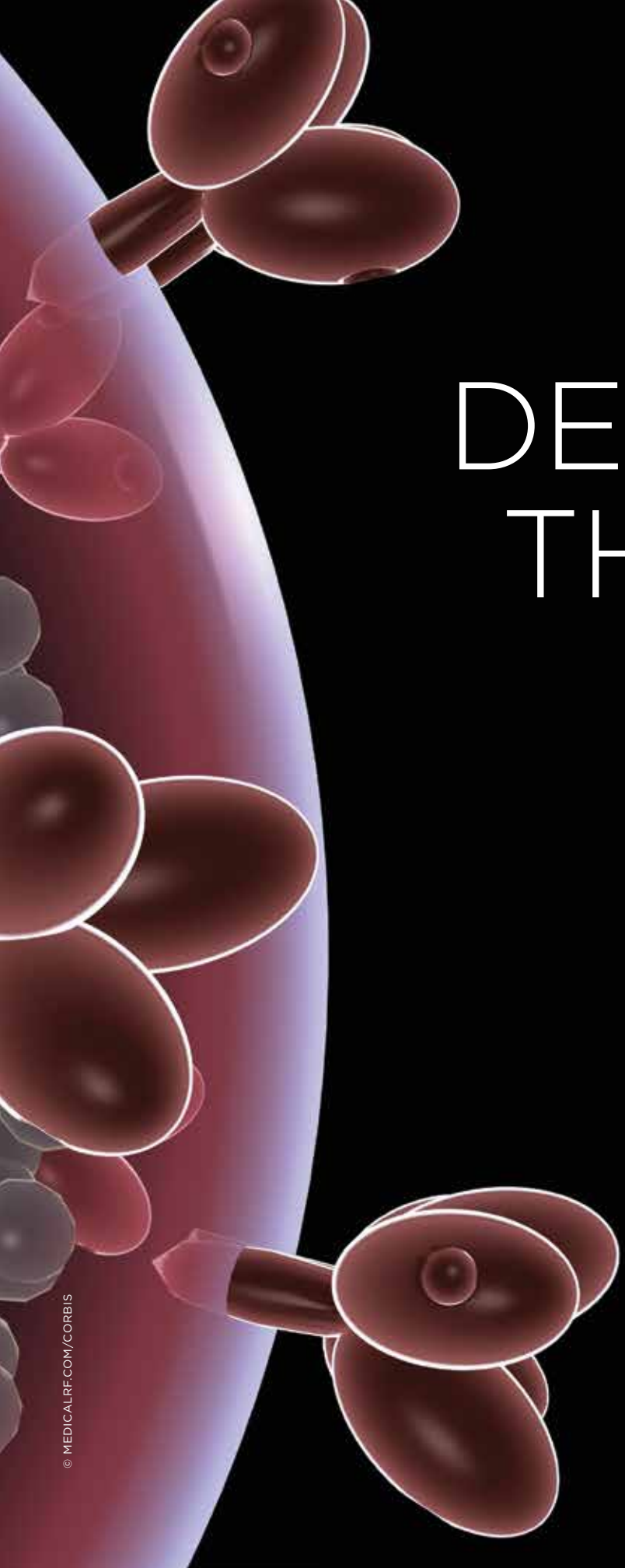






# DEFEATING THE VIRUS

Recent discoveries are spurring a renaissance in HIV vaccine research and development.

BY WAYNE C. KOFF

Measles is back. After declaring it eliminated in 2000, the United States is now dealing with an uptick in cases. The latest outbreak began in California's Disneyland theme park last December and, by early April, had ballooned to 159 cases across 18 states, according to the US Centers for Disease Control and Prevention (CDC). This troubling situation serves as a stark reminder of the importance of immunization, which for many years had kept this once ubiquitous and sometimes deadly childhood disease in check. Developed in the late 1960s under the guidance of longtime Merck scientist Maurice Hilleman, the two-dose regimen of the combination vaccine against measles, mumps, and rubella (MMR) is estimated to be 97 percent effective at preventing measles in a vaccinated individual.

But developing vaccines against measles and other diseases was relatively straightforward when compared with the process scientists must use today to fight much more complex pathogens, such as HIV. Part of what makes HIV such a difficult virus to combat is its ability to furiously mutate the trimeric envelope protein on its outer surface. This high mutation rate gives rise to multiple HIV subtypes that circulate around the globe, allowing the virus to escape the responses that human immune systems mount against it. The approach that proved so successful for the MMR vaccine—using attenuated versions of the pathogens to immunize people—isn't feasible for HIV because of concerns that the virus could mutate and regain its pathogenicity. And using a killed or inactivated virus—the other classic approach of vaccinology, used

to develop vaccines against polio and influenza viruses, among others—doesn't effectively address the unprecedented genetic variability of HIV.

There are other challenges unique to HIV. Quickly following transmission, the virus disseminates and establishes a persistent infection, including hidden reservoirs from which it can strike again at any time. (See "Hidden Menace" on page 34.) The opportunity for a vaccine-induced response to prevent infection or to control the initial, limited infection is thus short-lived. And while many people mount an effective immune response to and recover from most other viral infections, not a single person infected with HIV has cleared the virus on his or her own. The lone individual considered cured of HIV—Timothy Ray Brown, also known as the Berlin patient—only reached this milestone after receiving two bone marrow transplants to treat acute myeloid leukemia, which he'd developed after a decade of living with HIV and taking antiretroviral drugs. Doctors deliberately chose a stem cell donor with a genetic mutation that is known to confer resistance to HIV infection, in addition to a panoply of other chemotherapies and immune suppressive treatments to treat his acute myeloid leukemia. Attempts to repeat the success of this complex approach in other individuals with both cancer and HIV have so far been futile.

Scientists still don't understand how to elicit specific, durable, and protective immune responses against HIV. Animal models, while informative, can only hint at what works. This means HIV vaccine researchers need to be as wily as the virus we are trying to combat. Progress during the past five years is spurring creative and promising new approaches. Armed with intriguing results from clinical trials and tremendous progress in isolating and understanding the evolution of broadly neutralizing antibodies against HIV, the field is now poised to elucidate the rules of immunogenicity and accelerate progress toward an effective vaccine.

Success cannot come too soon. Despite considerable advances in preventing new HIV infections and in delivering lifesaving treatment to those already infected, 2.1 million people worldwide contracted HIV in 2013, according to the Joint United Nations Programme on HIV/AIDS (UNAIDS). In the same year, some 1.6 million people died of HIV/AIDS or related complications. Altogether, since it was identified in 1983, HIV has infected 78 million people and killed half of them. An effective vaccine is a critical component to ending the morbidity and mortality caused by the disease.

## Clues from trials

The HIV vaccine field has had its fair share of disappointing results from large, late-stage clinical trials. In 2007, vaccinations were stopped in the STEP and Phambili trials of a vaccine candidate that used replication-defective adenovirus serotype 5 (Ad5) to deliver HIV antigens designed to induce cellular immune responses against HIV. Then in 2013, vaccinations were terminated in the HVTN 505 trial, which tested a different Ad5 candidate in a prime/boost combination with a DNA-based vaccine. All three candidates failed to prevent HIV infection or blunt the disease's course in those who became infected.

But in 2009, the field did get a first, albeit modest, clinical signal for feasibility of an HIV vaccine in humans, when scientists at the US Military HIV Research Program (MHRP) reported that a prime/boost combination of two different vaccines reduced the rate of HIV infection by 31.2 percent in more than 16,000 volunteers in Thailand.[1,2] That trial, known as RV144, tested the canarypox virus–vectored vaccine candidate ALVAC-HIV, followed by a modified HIV gp120 protein subunit vaccine named AIDSVAX gp120 B/E, which had provided no protection in previous efficacy trials when administered on its own.

Researchers are working to determine the immune responses that led to this modest level of protection. Meanwhile, further insights may come from a new round of clinical efficacy trials for this prime/boost combination. Expected to begin in South Africa in late 2016, the new trials are designed to evaluate modifications to the vaccine candidates and regimen, including testing related HIV immunogens, different adjuvants, and new immunization schedules with additional booster shots intended to improve both strength and durability of immune responses.

**Developing vaccines against measles and other diseases was relatively straightforward when compared with the process scientists must use today to develop vaccines against much more complex and difficult pathogens, such as HIV.**

## Going broad

Researchers widely agree that an ideal HIV vaccine would induce the production of so-called broadly neutralizing antibodies, which are capable of neutralizing a broad swath of HIV strains and are produced naturally by approximately 25 percent of chronically HIV-infected people. To accomplish this, researchers must first identify what immunogens can elicit such a response. Although this remains a challenge, some scientists are making significant progress by employing reverse-engineering or structure-assisted vaccine discovery. This new approach starts with isolating broadly neutralizing antibodies from chronically infected HIV patients whose immune systems produce them. Researchers can then identify an antibody's target on the virus, use the molecular structure of this target site to design immunogens that mimic these sites, and immunize volunteers with these mimics to try to elicit the desired antibody response.

HIV vaccine researchers were buoyed recently by promising results from the use of this structure-based design strategy to produce a vaccine candidate against pediatric respiratory syncytial virus (RSV), which is the leading cause of hospitalization for

PUTTING VACCINES TO THE TEST: A recently launched clinical trial in South Africa investigates an HIV vaccine regimen based on the promising RV144 study, which showed a 31.2 percent reduction in HIV infection rates in volunteers receiving a prime/boost combination of two different vaccine candidates. The South African trial, called HVTN 100, has been adapted to the HIV subtype that predominates in the region.

children under five years of age worldwide. Peter Kwong and colleagues at the Vaccine Research Center (VRC) of the US National Institute of Allergy and Infectious Diseases first identified a site on an RSV envelope glycoprotein that extremely potent neutralizing antibodies target before the virus fuses with the host cell membrane. The researchers then identified and incorporated a series of mutations to stabilize the RSV protein in this conformation, engineered a version of the target site, and used it to immunize mice and rhesus macaques, eliciting high titers of neutralizing antibodies against RSV in both species.[3] Similar results were also observed after vaccination with computationally derived RSV proteins.[4]

With these proof-of-principle studies demonstrating the effectiveness of this approach, coupled with recent advances in identifying HIV-specific broadly neutralizing antibodies, HIV vaccine researchers are now working to apply these principles to design and screen new vaccine candidates. In 2009, a consortium of research institutions reported the isolation of two potent broadly neutralizing antibodies from an HIV-infected donor who was part of a large cohort study led by the International AIDS Vaccine Initiative (IAVI), where I serve as chief scientific officer. These new antibodies neutralized HIV at 10- to 100-fold lower concentrations than the previously identified antibodies and were effective against a broader swath of viruses.[5] This finding kicked off a flurry of new antibody discoveries, leading to the isolation of hundreds of HIV-specific broadly neutralizing antibodies, many targeting a relatively small number of specific sites on the virus. Characterization of these target sites has led to identification of the molecular structures of at least four highly conserved regions on HIV's envelope protein that can now be used to design vaccine immunogens. (See illustration on page 44.)

This boon in antibody isolation and characterization represents a major advance for structure-based HIV vaccine design efforts. Encouragingly, these antibodies can protect monkeys from infection with a hybrid simian/human immunodeficiency virus (SHIV), suggesting that a vaccine capable of inducing them in humans may afford protection against HIV.

Another major advance toward developing an effective HIV vaccine came in 2013 when a team of researchers led by John Moore at Weill Cornell Medical College in New York City and Ian Wilson at the Scripps Research Institute in La Jolla, California, obtained an atomic-level image of the HIV envelope trimer, the principal target for broadly neutralizing antibodies.[6,7] To capture this detailed image, the researchers first had to engineer a more stable form of this notoriously unstable protein, then use cryo-electron microscopy and X-ray crystallography to reveal its structure. A high-resolution structural model of the pre-fusion, closed form of HIV envelope by Kwong and colleagues at the VRC soon followed.[8] The vaccine field had been stymied for years by failed efforts to stabilize HIV's floppy surface protein. But with these detailed structures now in hand, a stable HIV envelope trimer that itself may be useful as a starting point from which to design an immunogen, and a suite of newly identified, conserved viral epitopes, scientists are entering a new phase of vaccine design.

## No ordinary antibodies

At the same time that researchers are identifying potential vaccine immunogens to elicit broadly neutralizing antibodies, there is also a renewed focus on understanding how these potent antibody responses develop naturally in chronically HIV-infected individuals. Researchers are trying to determine how the virus or a vaccine immunogen can direct the immune system to make antibodies that recognize the highly conserved HIV epitopes. By tracking the arms race that occurs between virus and immune system in the course of natural infection, researchers have found that neutralizing antibody responses don't appear until several months after HIV infection occurs, by which time the virus has

BROOKE AUCHINCLOSS

## VACCINATING AGAINST HIV

The strategies that have been used to develop most of today's successful vaccines—using attenuated, killed, or inactivated pathogens—don't work for HIV, which boasts unprecedented genetic variability and a high mutation rate. Researchers are now testing a number of tactics in parallel to protect people against the wide range of HIV subtypes that continue to infect the human population.

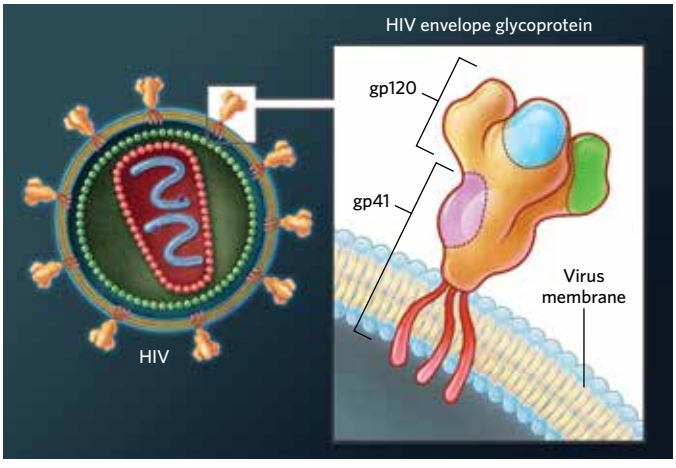
### CHARACTERIZING ANTIBODY FORMATION

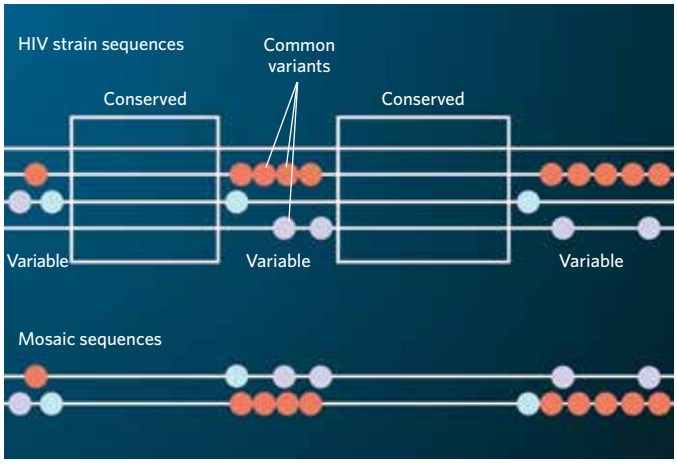
About 25 percent of chronically HIV-infected people naturally produce antibodies that are capable of neutralizing a broad swath of HIV strains by targeting conserved regions (blue, green, and pink) of the virus's envelope glycoprotein. Researchers are now isolating these so-called broadly neutralizing antibodies and identifying potential vaccine immunogens that may elicit such defenses, a strategy called structure-assisted vaccine discovery. Some groups are experimenting with delivery of multiple immunogens in a specific sequence, to mimic the natural process observed in chronically infected individuals.

### MOSAIC ANTIGENS

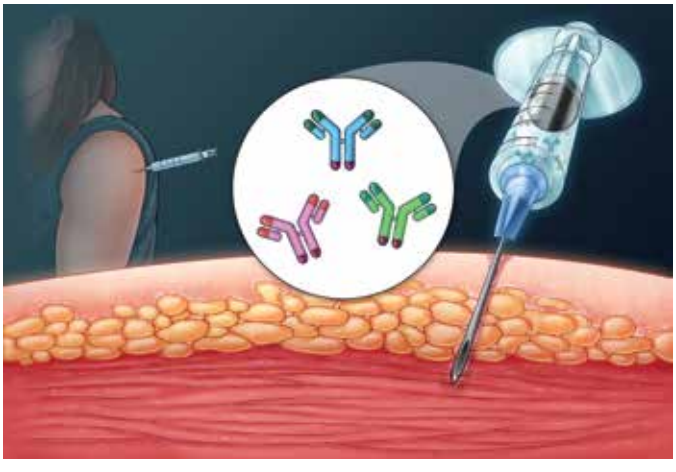
Computationally derived proteins known as mosaic antigens are created by stitching together stretches of DNA from a range of HIV variants. They can be delivered via viral vector to elicit a cellular immune response, inducing the activity of $CD4^+$ T cells that can boost the potency and durability of broadly neutralizing antibodies and activating cytotoxic $CD8^+$ T cells to help control HIV infection.

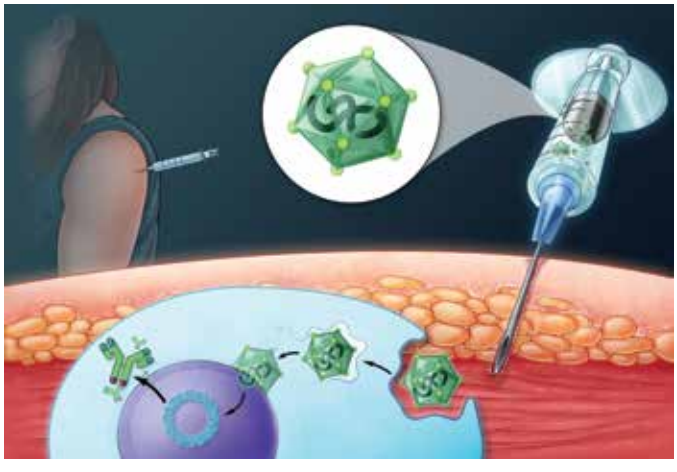
### INDUCING IMMUNE RESPONSES




### DELIVERING ANTIBODIES





### PASSIVE IMMUNOPROPHYLAXIS

As an alternative to coaxing the immune system to generate broadly neutralizing antibodies, scientists are also testing whether these molecules can be delivered directly to HIV-positive individuals, an approach called passive immunization. Research is ongoing to increase the potency and/or half-life of these antibodies and to improve function of the antibody's Fc portion, which interacts with immune cells that lyse infected cells.

### GENE TRANSFER

Yet another possibility is to use viral vectors to deliver the genes encoding such antibodies. Researchers are also engineering viral vectors to express mutated versions of the coreceptors that HIV uses to infect host cells, thereby inhibiting viral entry without having to elicit a lengthy and complex antibody maturation process. This approach has proven successful in protecting rhesus macaques from simian/human immunodeficiency virus (SHIV) infection.

mutated enough that these responses are unable to adequately control viremia. Antibody responses that can neutralize HIV more broadly—the type researchers seek to elicit with a vaccine—appear only after two or more years of chronic HIV infection.

Careful examination of the antibodies themselves also indicates these are no ordinary antibodies. Many have variable regions with unusually high levels of somatic hypermutation, the process by which B cells accrue genetic mutations that lead to an improved affinity for the pathogen. This high hypermutation suggests that B cells giving rise to these broadly neutralizing antibodies go through several rounds of mutation and selection in response to chronic exposure to HIV proteins, which could also be why they take so long to appear and then only in a subset of HIV-infected people.

In this context, developing a vaccine to elicit broadly neutralizing antibodies is a formidable task indeed. The vaccine will have to guide the immune system to do something it only sometimes accomplishes in natural infection, and do so in a fraction of the time. This will likely require some coaxing. One method, championed by Bart Haynes of Duke University, involves administering a series of HIV envelope immunogens in a set sequence to elicit antibody maturation that mimics the natural process observed in chronically infected individuals. This sequential immunization strategy is currently being tested in monkeys, and may soon advance to human trials.

Bill Schief and colleagues at IAVI's Neutralizing Antibody Center at Scripps in La Jolla are testing another approach that involves starting with a computationally derived HIV immunogen that can bind multiple broadly neutralizing antibodies and their precursors.[9] This immunogen is presented on nanoparticles that can be used as a priming vaccination to kick off the process of somatic hypermutation. Eliciting fully matured, neutralizing antibodies, however, will likely require additional boosting with different immunogens along the way that are more representative of native HIV epitopes.

This is uncharted territory, and ideally the best of these designer immunogens will be tested in a series of human trials to better define the rules of immunogenicity and to develop a vaccine that most effectively exploits those rules.

## Bypassing the immune system

In the absence of immunogens capable of eliciting neutralizing antibodies against HIV, researchers are also exploring whether direct injection of HIV-neutralizing antibodies may be an efficient means of preventing HIV infection. This so-called passive immunization approach is now in Phase 1 clinical trials involving both HIV-positive patients and uninfected volunteers to determine the safety and pharmacokinetics of these vaccines. Early data from monkey studies suggest that such direct injection of broadly neutralizing antibodies may also have therapeutic benefits or even be part of a multifaceted HIV cure strategy.[10]

Meanwhile, work is underway to optimize the antibodies used for such passive immunization by introducing mutations that increase their potency and/or half-life and by improving the function of the antibody's Fc portion, which can interact with monocytes and natural killer cells to lyse virus-infected cells. There are also plans to study a cocktail of antibodies in passive immunization studies to increase the breadth of activity against HIV.

Yet another approach to circumvent the immune system's role in making broadly neutralizing antibodies is to use an adeno-associated-virus (AAV) vector to deliver the genes encoding such antibodies into cells, which could then express the antibodies. Philip Johnson of the Children's Hospital of Philadelphia pioneered this approach and now, in collaboration with IAVI, is testing an AAV1 vector carrying the genes for the broadly neutralizing HIV antibody PG9 in an ongoing Phase 1 trial. If it is successful, a cocktail of vectors carrying two or more broadly neutralizing antibodies will be tested. Similarly, David Baltimore of Caltech is using another AAV vector (AAV8) to deliver a broadly neutralizing HIV antibody targeting the virus's CD4 binding site, an approach that was effective in protecting humanized mice from mucosal HIV transmission.[11]

Antibody responses that can neutralize HIV more broadly—the type researchers seek to elicit with a vaccine—appear only after two or more years of chronic HIV infection.

In addition to expressing the genes for broadly neutralizing antibodies, AAV vectors may also be engineered to express synthetic proteins that prevent the virus's entry into host cells. Michael Farzan of Scripps in Florida and colleagues recently demonstrated the success of this approach in preventing SHIV infection in rhesus macaques.[12] Collectively, these efforts are paving the way for a nontraditional immunoprophylaxis that can protect against HIV infection without depending on the lengthy and complex antibody maturation process required to generate broadly neutralizing antibodies through immunization.

## Cellular immunity

In parallel with studies focused on eliciting broadly neutralizing antibodies, scientists continue to pursue strategies to elicit cell-mediated immune responses against HIV. Induction of $CD4^+$ T cells can boost the potency and durability of broadly neutralizing antibodies and also help activate robust cytotoxic $CD8^+$ T cells aimed at controlling HIV infection. But, as with effective antibodies, such cellular immune strategies face the challenge of high levels of genetic diversity among circulating HIV subtypes.

Bette Korber and colleagues at Los Alamos National Laboratory are designing so-called mosaic antigens to overcome HIV diversity. These are computationally derived proteins created by stitching together genetic sequences from across the entire HIV genome. (See illustration on previous page.) These mosaic anti-

gens, when delivered via viral vectors either alone or in combination with each other or a protein booster component, can provide greater breadth of cellular immune responses against HIV variants and protect against SHIV infection in monkeys.[13] Researchers recently initiated Phase 1 trials of this approach.

An alternative tactic for tackling the variability of HIV is to focus on eliciting cellular immune responses to the most conserved regions of the HIV proteome, an approach championed by Andrew McMichael and Tomas Hanke of Oxford University. Most recently, mosaic antigens that are focused solely on these conserved regions of HIV were designed to optimize coverage of such immunogens across HIV's global diversity. These conserved mosaic antigens are undergoing preclinical testing.

Lastly, researchers are harnessing the unique qualities of cytomegalovirus (CMV) that evoke robust and broad cellular immune responses by using this virus as a vector for HIV vaccine development. In monkey studies spearheaded by Louis Picker of Oregon Health & Science University, administration of the rhesus form of cytomegalovirus (RhCMV) expressing proteins from simian immunodeficiency virus (SIV), the monkey equivalent of HIV, led to durable control of SIV infection following challenge, including evidence of complete clearance of pathogenic SIV infection in some animals.[14] The precise mechanism for this protection remains unknown, but effector memory T-cell responses appear to play a role. Picker and colleagues are now developing a prototype CMV vector to assess safety and immunogenicity in humans. This approach should advance to clinical testing by 2016.

## Beyond HIV

Although many challenges remain, the development and deployment of a safe and effective HIV vaccine is an urgent global health priority. Recent progress is reinvigorating vaccine discovery efforts, and research to better understand HIV and the immune response against it will help to inform broader vaccine efforts. Already, researchers have identified broad and potent neutralizing antibodies against influenza, dengue, hepatitis C, and other complex pathogens. And investigators are applying structure-based vaccine discovery to a wide spectrum of infectious diseases for which vaccines are still needed.

Similarly, new technologies of genetic and immune monitoring and of systems biology, coupled with novel strategies for induction of cellular immune responses, are being applied for development of prophylactic and therapeutic vaccines against infectious diseases and cancers. The prospect of decoding the immune system and unravelling the rules of immunogenicity in humans now offers the potential to usher in a golden age of vaccinology that will relegate HIV and other modern global killers to the same fate as the childhood diseases of the 1950s that are now easily prevented through vaccination. ■

*Wayne C. Koff is the chief scientific officer at the International AIDS Vaccine Initiative (IAVI).*

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# Hearts on Trial

As researchers conduct the most rigorous human trials of cardiac cell therapies yet attempted, a clear picture of whether these treatments actually work is imminent.

BY KERRY GRENS

For the better part of an hour, the catheterization lab at Cedars-Sinai Medical Center in Beverly Hills bustles like a restaurant kitchen on a Friday night. Nurses pivot carefully around a table laden with sterile instruments while a trio of physicians fusses over a catheter inserted into the groin of cardiac patient Ken Anderson. On a large screen next to his bed, the video feed from a tiny camera at the tip of the catheter shows the tube passing through Anderson's vasculature into a coronary artery. Amidst the hubbub, only the patient is still, occasionally answering questions from a nurse about his level of comfort.

All at once, everything stops. The nurses stand still; the doctors stop chatting; all eyes turn to the image of Anderson's beating heart.

Michelle Domingo, the study coordinator for the experimental cell therapy Anderson is about to receive, counts down from five, then shouts: "Inject!" For the next five minutes a doctor very, very slowly presses the plunger on a syringe hooked up to the catheter, sending 10 mL of solution and millions of stem cells into Anderson's heart.

The cells, called cardiosphere-derived cells, originated from an organ donor whose heart was not suitable for transplant. Anderson, who had refused a heart transplant, flew to Los Angeles from Missouri to take part in this experimental treatment, hoping it would help his damaged heart become more effective at pumping blood. "I'm an old guy," says the 69-year-old, speaking a few weeks later from his office at the surgical supply company he continues to run, despite his impaired cardiac function. "There are people in their thirties and forties who need hearts. . . . Why should they put a heart in me?"

Anderson is one of thousands of patients who have had cells injected into their hearts over the past 10 years. (See "Trial of the Heart," *The Scientist*, October 2006.) Like any new branch of medicine, cardiac cell therapy has progressed in fits and starts. Despite dozens of clinical trials, there's no slam-dunk treatment for improving the cardiac function of heart failure patients, but marginal, statistically significant improvements observed in some of the studies are propelling the cell-based therapies to ever larger, more expensive, and more rigorous trials. Most cardiologists remain underimpressed, says Anthony Mathur, the director of cardiology at Barts Health NHS Trust and the head of an ongoing Phase 3 trial in Europe that involves injecting bone marrow cells into heart attack patients. "If [the trial] is positive, that's great, but I still think we'll have quite a challenge to convince people," he says. "If it's negative, then you get most of the cardiac community saying, 'Yep, we expected that.' "

Some anticipate that the results of ongoing Phase 3 trials will finally provide definitive evidence supporting the efficacy of cell therapies for the heart. On the other hand, negative results could spell the end of the approach altogether.

Now, it's make or break. Some anticipate that the results of Mathur's trial and two other ongoing Phase 3s will finally provide definitive evidence supporting the efficacy of cell therapies for the heart—evidence that has so far been lacking. On the other hand, negative results could spell the end of the approach altogether. "If our Phase 3 doesn't work, I think there's little likelihood any program could succeed in this indication," says Christian Homsy, the CEO of Cardio3 Biosciences, a Belgium-based firm sponsoring a clinical trial involving bone marrow–derived cells. "In the event they don't work [this time], I think it will be the end."

## Mysterious mechanism

The cardiac-derived progenitor cells Anderson received are in the earliest phases of human testing. But the cells used in the therapies that are now moving into Phase 3 trials all originate in the bone marrow, and include mesenchymal stem cells as well as other cell types. (See illustration on opposite page.) The idea to pluck cells from a person's bone marrow and shoot them into the heart took root in 2001, when Piero Anversa's group, then at New York Medical College, showed that doing so in mice could help regenerate damaged heart tissue.[1] Within just a few months, patients in Germany were undergoing the procedure. "It was an example of very, very rapid translation of something that was an animal finding into clinical trials," says Jalees Rehman, a regeneration researcher at the University of Illinois at Chicago College of Medicine.

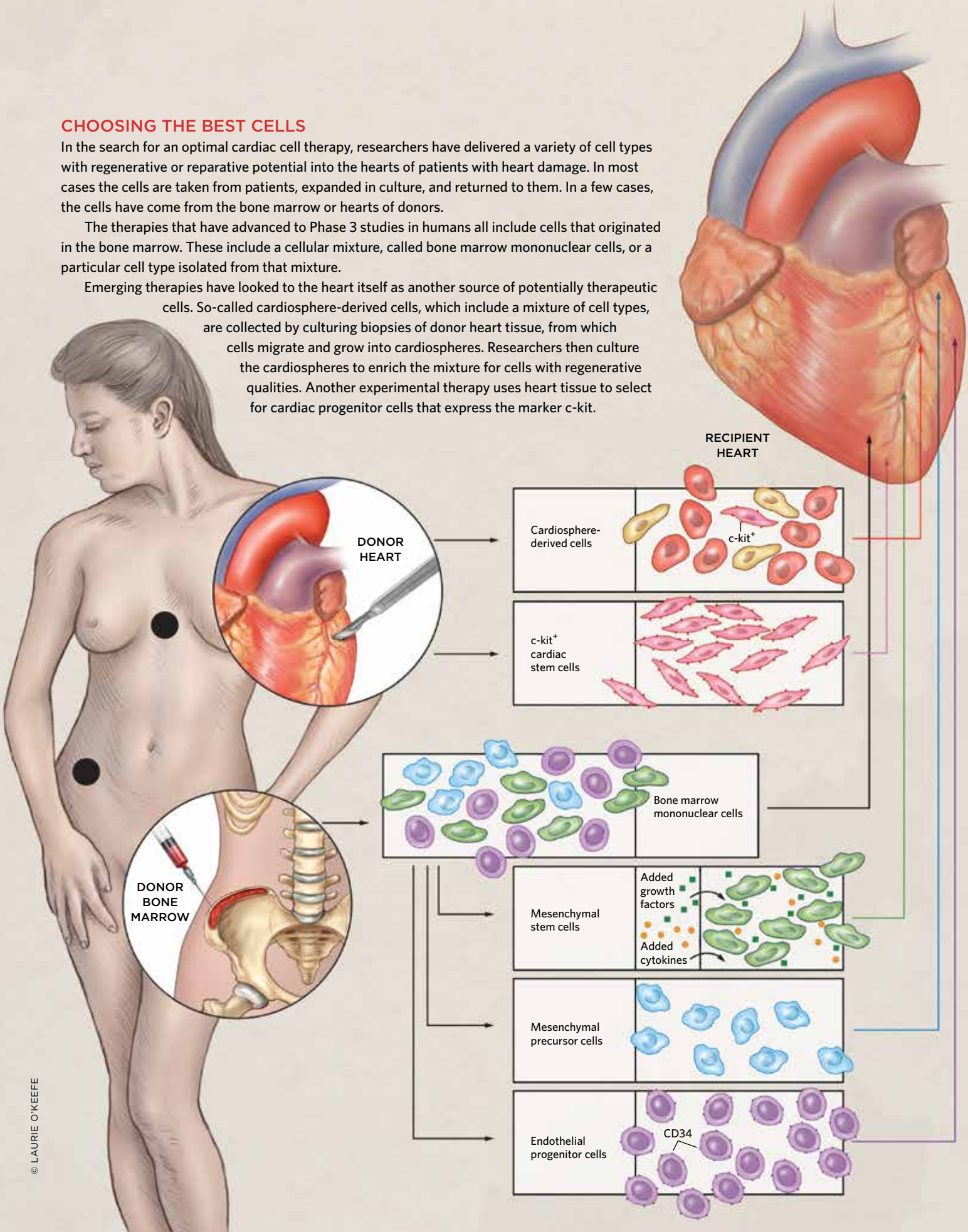
Yet no one knew how the cells worked. At the time, the prevailing thought was that stem cells took up residence in the heart and proliferated to produce new tissue. But this idea has since become a matter of debate. While some researchers claim the cells can form new cardiac muscle, others assert that the cells only very rarely differentiate into cardiomyocytes and instead support cardiac regeneration by other means. Some studies have found, for example, that "any trace of the cells is gone after four weeks," says Eduardo Marbán, the lead scientist at Cedars-Sinai who developed the heart-derived cell therapy that Anderson received. "We had to consider the possibility that the cells were not acting in the way we thought they were."

## CHOOSING THE BEST CELLS

In the search for an optimal cardiac cell therapy, researchers have delivered a variety of cell types with regenerative or reparative potential into the hearts of patients with heart damage. In most cases the cells are taken from patients, expanded in culture, and returned to them. In a few cases, the cells have come from the bone marrow or hearts of donors.

The therapies that have advanced to Phase 3 studies in humans all include cells that originated in the bone marrow. These include a cellular mixture, called bone marrow mononuclear cells, or a particular cell type isolated from that mixture.

Emerging therapies have looked to the heart itself as another source of potentially therapeutic cells. So-called cardiosphere-derived cells, which include a mixture of cell types, are collected by culturing biopsies of donor heart tissue, from which cells migrate and grow into cardiospheres. Researchers then culture the cardiospheres to enrich the mixture for cells with regenerative qualities. Another experimental therapy uses heart tissue to select for cardiac progenitor cells that express the marker c-kit.



Many scientists now believe that the introduced cells perform a paracrine function, signaling the activation of reparative pathways via growth factors or other secreted messengers. On its own, the heart regenerates about one percent of its tissue per year via the division of cardiomyocytes; perhaps cell therapies simply boost that normal behavior. "The mechanism is still unclear," says cardiologist Roberto Bolli of the University of Louisville.

The absence of a concrete mechanism of action has been one of the main criticisms of the field. "I would like to be sure I understand the mechanism before I jump ahead to big trials," says Rehman. "Just because it works is not good enough."

On the other hand, most patients don't care how a treatment works, just that it does. And with no cure for heart damage yet available, patients like Anderson are willing to try experimental treatments. "We have more trials than we have meaningful basic science papers," says Richard Lee, a regenerative medicine researcher and cardiologist at Harvard Medical School. "You'd like it to be the other way round. But I understand why there was an explosion [of clinical trials]—because there is such a need."

**We have more trials than we have meaningful basic science papers.**

—Richard Lee, Harvard Medical School

And many researchers disagree that a known mechanism is required for advancing the therapy. "Nothing moves a field forward like actual clinical trials," says Joshua Hare, a University of Miami cardiologist who has conducted human studies using mesenchymal stem cells. While mechanisms are important—knowing them can help optimize treatments, for instance—"you can't slow things down because the mechanism of action isn't agreed upon by everybody."

## With bated breath

The three ongoing Phase 3 trials involve cells extracted from bone marrow—each treatment comprising its own particular cellular concoction. In the "BAMI" study, currently recruiting subjects in Europe, patients who have suffered a heart attack will receive an autologous mixture of various bone marrow cells, called mononuclear cells. Congestive heart failure patients enrolling in the congestive heart failure cardiopoietic regenerative therapy (CHART)-1 study in Europe and Israel will receive mesenchymal stem cells derived from their bone marrow. And in another congestive heart failure study in the U.S. and Canada, patients will receive mesenchymal precursor cells derived from the bone marrow of donors. These studies—all double-blind and placebo-controlled—are the most scientifically rigorous human experiments to date in the field of cardiac cell therapy.

The BAMI trial is the simplest of the three, with mononuclear cells extracted from the bone marrow delivered directly into the heart with no manipulation. Investigators will measure whether patients randomly assigned to receive the cell therapy have a reduced chance of dying over the next two years than patients receiving a placebo. "We have a high bar" for judging success, says Mathur, one that's designed to put an end to questions about whether or not the therapy works.

One of the criticisms of the BAMI approach is that the cellular mixture administered to patients contains very

## WAITING IN THE WINGS

In a building across the street from Cedars-Sinai hospital in Beverly Hills, a researcher sits hunched at a fume hood, carefully slicing up cardiac tissue into thin sections. In his gloved hands are pieces of the heart of a young woman who recently passed away. Her donated organ made it here to a laboratory at Capricor Therapeutics, the biotechnology firm that is sponsoring the trial Ken Anderson is participating in. But her heart cells won't go into patients. They will instead be used for preclinical research. One of the big questions the Capricor team would like to answer is which cells pack the most regenerative punch.

"It has been a very long journey of one disappointment after another . . . where the [clinical trial] results have been less than overwhelmingly positive," says Linda Marbán, Capricor CEO and wife of Cedars-Sinai's Eduardo Marbán. The reasons, she says, are that "the trials were not well powered, the endpoints were not well adjudicated, and . . . those particular cells may not be the right cells."

While researchers test the efficacy of bone marrow–derived cells in ongoing Phase 3 studies, waiting in the wings are alternative approaches that have shown promise in preclinical models or early-stage human testing. The cardiosphere-derived cells that Linda Marbán's team is studying, for example, are shed by cardiac tissue when a sample is put in culture (the cells form spherical clusters, hence the name). When infused into 17 heart attack patients in a Phase 1 study, the cells caused a reduction in scarring and regrowth of heart muscle. "Conceptually, it put the wind in our sails," says Eduardo Marbán. Although the treatment did not improve patients' cardiac function, Capricor is moving ahead with two other cell therapy trials, both using allogeneic cardiosphere-derived cells.

Another cell type derived from cardiac tissue, known as c-kit$^+$ cells, has also shown promise in an early-stage trial. Like cardiosphere-derived cells, c-kit$^+$ cells exhibit regenerative power when administered to the hearts of laboratory animals. And even further along in human studies are CD34$^+$ endothelial progenitor cells isolated from bone marrow. Researchers showed that these stem cells can reduce chest pain and increase patients' exercise capacity, and a Phase 3 study is planned.

Preclinically, other cell types are stirring up excitement. Charles Murry's lab at the University of Washington, for instance, made a splash last year after using human embryonic stem cells to

few stem cells and a slew of other cell types, including lymphocytes and monocytes. In the CHART-1 trial, based on the work of Andre Terzic at the Mayo Clinic in Rochester, Minnesota, mesenchymal stem cells are isolated from the patient's bone marrow cell mixture, then bathed in a cocktail of growth factors and cytokines to "preemptively upgrade the regenerative capacity" of the cells, says Terzic. The final batch of cells that are administered back into the patients "express certain markers of cardiac differentiation," says Homsy of Cardio3 Biosciences. The trial will track several outcomes, including exercise capacity and subsequent heart events, in addition to survival.

The third trial, sponsored by Teva Pharmaceutical Industries, also involves selecting a subset of cells from the bone marrow: mesenchymal precursor cells, called CEP-41750 cells, which are thought to aid in tissue repair and stimulate blood vessel growth. In this trial, the researchers are using cells collected from donated bone marrow, rather than from the patients themselves, paving the way to creating an off-the-shelf product.

Results from each of these trials are expected in the next one to two years. In the meantime, researchers are concocting even more refined versions of cardiac cell therapies, including the cells Anderson received, which are derived from donor hearts. Having yielded positive results in animal models and early-stage human trials, so-called "second-generation" cell therapies, employing other cardiac progenitor cells or select bone marrow cells, could replenish the field with fresh cell therapy approaches even if current Phase 3 studies fail. (See "Waiting in the Wings" below.) But which cell type or combination of cells will prove the most effective in treating heart damage remains to be seen. "I've gotten away from the notion there's an optimal cell type. We believe we can enhance the [regenerative] effect through cell mixtures," says Hare, who is launching a clinical trial to test a therapy that comprises both mesenchymal stem cells and cells taken from the heart.

## Benefit and bias

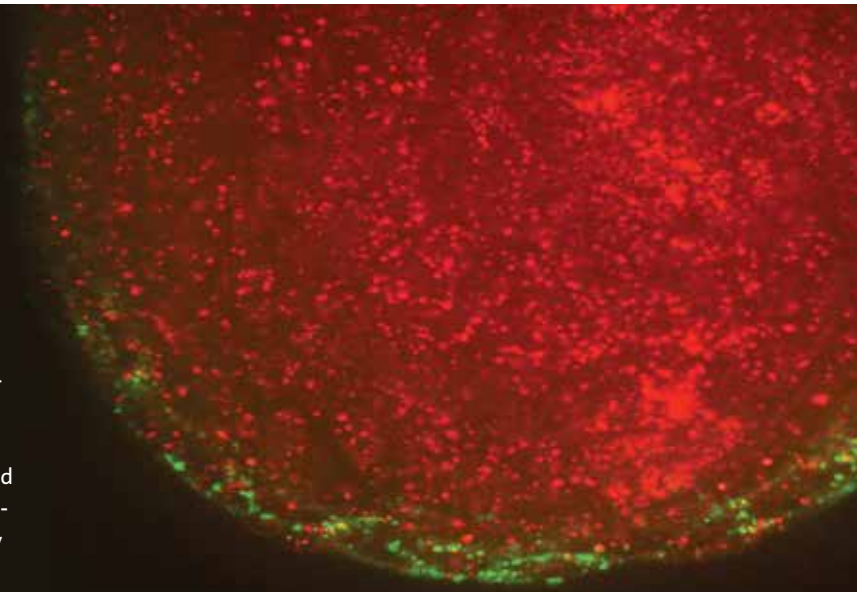
While early-stage research continues to hammer out which type of cell is optimal for repairing heart damage, a bigger question faces the field: Do cardiac cell therapies even work? Studies have found a range of results, from no benefit to substantial therapeutic effects, and research in the field has been plagued by a couple of high-profile scandals surrounding studies that made the biggest claims about heart regeneration. Chief among them has been the work of Bodo-Eckehard Strauer, a now-retired cardiologist from the University Hospital of Düsseldorf in Germany.

In 2001, just four months after Anversa's group published its success with administering bone marrow-derived cells to mice with damaged hearts, Strauer and his colleagues were the first to try the method on a human patient who had recently suffered a heart attack. Within a few months, they claimed, the patient's heart damage was reduced and his cardiac function improved. For a number of subsequent trials, Strauer continued to report positive results, but the claims were questioned by researchers who noted problems with data reporting, and upon investigating Strauer's work, the University of Düsseldorf concluded that there was evidence of scientific misconduct. Although none of Strauer's studies have been retracted,

successfully regenerate cardiac tissue in the damaged hearts of monkeys (*Nature*, 510:273-77, 2014). In this case, no one disputes the regenerative capacity of embryonic stem cells, but some have cautioned that their safety has not yet been sufficiently vetted to try them out in humans. Jalees Rehman at the University of Illinois at Chicago says cell therapy patches lined with regenerative cells such as induced pluripotent stem cells or embryonic stem cells are another possible route to cardiac regeneration.

If it turns out that the cells in clinical development aren't differentiating into new heart tissue, but instead are providing the support needed for existing cardiac cells to ramp up repair—as many now believe—there is the possibility that acellular approaches could effect regrowth and repair, without dragging along unnecessary cellular baggage. To this end, Eduardo Marbán is testing out the utility of exosomes—little membrane-bound bubbles filled with proteins and RNA—in the lab. Other teams are looking to introduce growth factors, cytokines, or genes to induce the heart to fix itself.

"As the field evolves, maybe we will not need the cell itself," says Andre Terzic of the Mayo Clinic. "That would be the ultimate product."



COURTESY OF CEDARS-SINAI HEART INSTITUTE

**FROM THE HEART:** Cells derived from cardiospheres (pictured here) express markers such as c-kit (red) and CD105 (green). These and other cell types are currently in preclinical and clinical development to improve cardiac function.

the field has largely turned its back on his work. Authors of recent meta-analyses of cardiac cell therapies, for instance, have opted not to include Strauer's reports.

More recently, the work of Anversa, now at Harvard, has come under scrutiny. Beginning last year, his lab has been the subject of an institutional investigation into potential scientific misconduct. One of his papers, describing the regenerative capacity of the human heart, has been retracted,[2] while another has received an Expression of Concern from journal editors at *The Lancet*, where it was published in 2011.[3]

Bolli says it will be unfortunate if the controversy over c-kit$^+$ cells spells their demise, because he believes the treatment is promising. He says the scandals in the field have given cardiac cell therapy a bad rap. "One or two bad apples make the whole field look bad," he says. "The best answer . . . is to look at the totality of the data."

But even studies that haven't drawn accusations of outright misconduct are riddled with problems, according to a recent review by Darrel Francis, a cardiology professor at the National Heart and Lung Institute at Imperial College London. Combing through 49 clinical trials of cardiac cell therapies, Francis and his colleagues exposed some 600 inconsistencies and reporting errors, including conflicts between figures and raw data, and impossible time lines—such as dead patients continuing to produce clinical data.[4] Later, Francis's team published a detailed list of problems with the report on Terzic's clinical trial of a mesenchymal stem cell therapy, resulting in multiple corrections.

**It has been a very long journey of one disappointment after another, where the clinical trial results have been less than overwhelmingly positive.**

—Linda Marbán, Capricor Therapeutics

The *Lancet* study included the positive results of a Phase 1 clinical trial called SCIPIO that delivered heart-derived cells known as c-kit$^+$ cardiac stem cells to patients with ischemic heart failure. The editors expressed concern regarding two supplemental figures, which Bolli—who ran the clinical trial at the University of Louisville's School of Medicine—claims had nothing to do with the reported improvements in patient outcomes. According to Bolli, Anversa's team prepared the cells for delivery into patients and sent them to Bolli for the infusions. "There was a clear division of labor," he says, and the supplemental figures in question have to do with the cell characterization, not patient outcomes. (Anversa declined to comment.)

The future of clinical trials on c-kit$^+$ cells is unclear, with no immediate plans to start a Phase 2 study, Bolli says. In addition to the concern surrounding the Phase 1 trial, c-kit$^+$ cells are at the center of the debate over the mechanism of action of cardiac cell therapies. While Anversa has published work that he claims illustrates these cells' ability to generate substantial amounts of new cardiac muscle, a number of researchers have produced evidence disputing this.

Interestingly, the number of discrepancies Francis found in a clinical trial correlated with the reported benefit of the treatment: those that showed the greatest effect of the cells had the most problems, while the trials with the best reporting found little therapeutic benefit. Francis says that poring over the cardiac cell therapy studies has soured him on the approach, and he expects that none of the therapies currently being studied will pan out.

Several other meta-analyses have also yielded cautionary results. A study published by Marbán and colleagues this year, for example, examined the results of 12 trials (not including Strauer's) that injected cells—mostly extracted from the bone marrow, with one study infusing cardiosphere-derived cells—into the hearts of patients following heart attack, finding no overall benefit.[5] But another meta-analysis of 31 clinical trials, from Mathur and colleagues, found a lower risk of dying or returning to the hospital for heart problems among heart-failure patients who received a bone marrow-derived cell treatment.[6] Mathur and his coauthors point out, however, that the studies they analyzed carry a high risk of bias—some were not blinded, for instance, and others didn't fully report patient outcomes.

Despite the concerns, many researchers believe it's important to continue with the Phase 3 studies, which may be the only way to get a solid answer out of a muddy mess of data. "I think BAMI will either be the end of bone marrow trials or the vindication of bone marrow trials," says Marbán. Even Francis, perhaps the most skeptical among cardiac cell therapy critics, agrees there's a learning opportunity to be had from the efficacy studies. "Maybe these carefully conducted Phase 3 trials will be positive, which will be excellent news for patients and a surprise to me. But if they are negative, then I think we are failing as a clinical scientific community if we do not thoroughly explore how it is that we got our entire clinical research field into this colossally expensive dead end." ■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# The Literature

GENETICS & GENOMICS

## Looking for Latent HIV

THE PAPER
L.B. Cohn et al., "HIV-1 integration landscape during latent and active infection," *Cell*, 160:420-32, 2015.

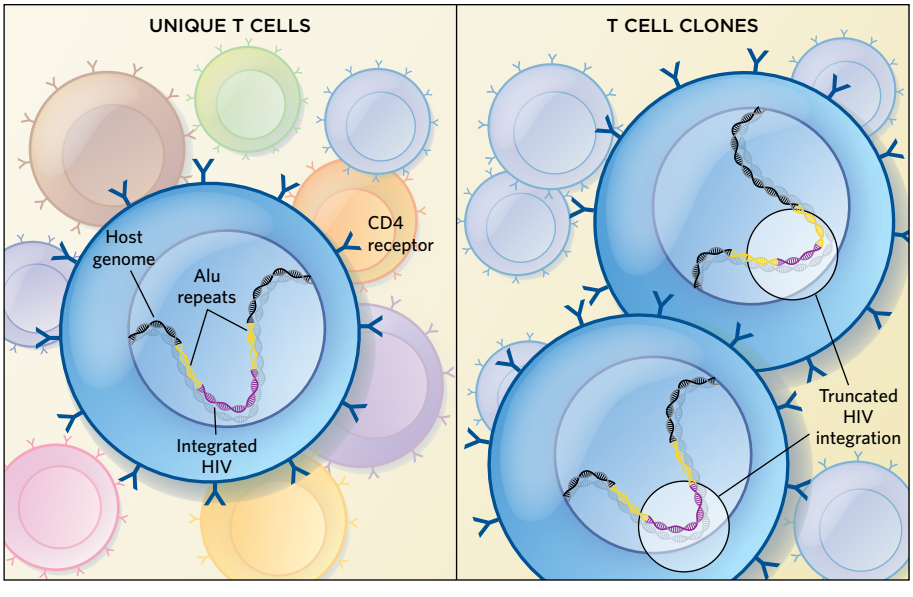
Antiretroviral treatments have transformed HIV infection from a death sentence to a manageable, though lifelong, condition. But remove the drugs that prevent the virus from replicating, and the infection comes roaring back. Although scientists have long known that somewhere in the bodies of HIV-positive people a latent reservoir of virus lies in wait, ready to replicate when conditions are right, researchers have struggled to locate it. One of the more popular latent-reservoir hypotheses is that the virus integrates into the genome of a memory T cell. That cell then divides many times, creating clones of the original integration that serve as an on-demand virus-making factory.

To test this idea, Lillian Cohn, a graduate student in Michel Nussenzweig's group at Rockefeller University, and colleagues amplified and sequenced integrated viral DNA (provirus) from patients' T cells to determine whether each integration was unique or a copy among many cloned cells. Cohn and her colleagues probed the sequences of 75 clones, only to discover that none appeared to have an intact copy of the viral genome. Many seemed to have missing or broken 5' untranslated regions, and the remainder had either undetectable or heavily mutated viral sequences. "That was unexpected," Cohn says. "While we didn't prove completely that the [viral integrations in the] clonally expanded cells are defective, we definitely did make a good case that the majority of the largely expanded clones are inactive."

Although the researchers may not have found the main source of the latent HIV reservoir, they did notice an interesting pattern in where the virus integrated into the T cells' genomes. Many of the integrations occurred in stretches of DNA with a particular retrotransposon sequence known as an Alu repeat. This DNA mark might indicate genomic regions that can tolerate viral integration, although this remains to be studied, Cohn says.

It's still possible that some intact viral DNA may be hidden in the clonally expanded population, the authors note in their paper. Lisa Frenkel, a University of Washington–based infectious disease researcher who has studied the integration of HIV into cancer gene loci, agrees. She cites recent evidence of an AIDS patient whose clonally expanded T cells produced detectable levels of virus in the patient's bloodstream, suggesting that clonal cells can, at least on occasion, hide the latent virus reservoir. Her own work has found that HIV harboring large deletions can still replicate itself. "When you make 1 [billion] to 10 billion new virions a day and they all have errors, it's a huge undertaking to really investigate these questions thoroughly," Frenkel says of the effort to find the definitive source of the reservoir. "It's kind of a needle in a haystack."

Determining whether single integrations are the primary source of most latent reservoirs awaits an update of Cohn's technique, which currently doesn't allow for the characterization of uniquely integrated viruses. The hunt for the source of the latent viral reservoir is still underway. —Jenny Rood




BUTTING IN: HIV integrates genetic material into the genome of a host's memory T cells, frequently at sites of so-called Alu repeats (below, left panel). Although these cells are suspected of harboring intact, latent viral DNA, researchers have found that clones of an infected T cell have short, dysfunctional integrations and thus likely do not compose the HIV reservoir that can replicate if a patient stops antiretroviral therapy (below, right panel).

**TRANSMITTER:** Western lowland gorillas appear to be the source of a particular type of HIV that has infected 100,000 humans.

MICROBIOLOGY

# The Origins of O

**THE PAPER**
M. D'arc et al., "Origin of the HIV-1 group O epidemic in western lowland gorillas," *PNAS*, doi:10.1073/pnas.1502022112, 2015.

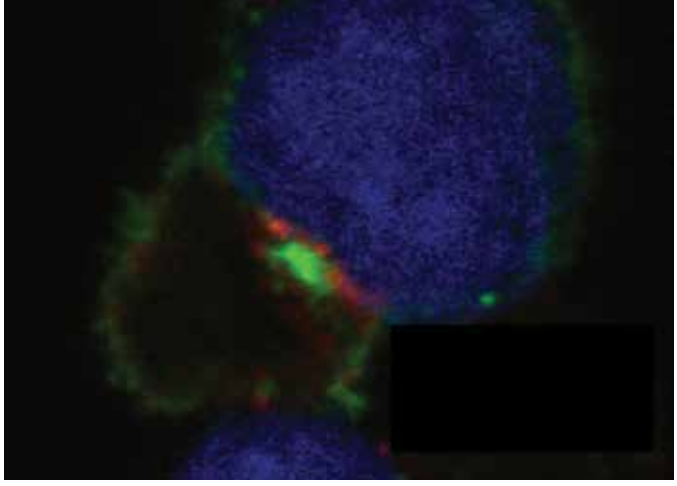
**THE STRAINS**
HIV jumped from apes to humans at least four times, as evidenced by genetically distinct groups of the virus that have been detected: M, N, O, and P. While N and P have had little impact, M is responsible for the pandemic affecting millions of individuals, and O has infected another 100,000.

**THE ORIGIN**
The M group of HIV-1 came from chimpanzees, likely in Cameroon. To uncover the roots of group O, Martine Peeters at INSERM and the University of Montpellier in France and colleagues trekked into the forests of central Africa to collect and analyze fecal samples from chimps and, while they were at it, from gorillas, too. "It became clear the O group is most closely related to the gorilla virus," says Peeters's collaborator Beatrice Hahn of the University of Pennsylvania.

**THE CHINK IN THE ARMOR**
Phylogenetic comparisons of various simian immunodeficiency viruses (SIVs) point to a gorilla having caught the virus from chimps. To understand how, the researchers scrutinized an antiviral protein, APOBEC3G, in gorillas. They found that it was vulnerable to counteractivity by a gorilla SIV protein, Vif, but not to Vif from chimp SIV. "The Vif protein must have changed, because it can overcome" APOBEC3G, says Welkin Johnson, a virologist at Boston College. "It's a direct example of how this APOBEC3G protein must be a genetic barrier to viruses jumping between species."

**THE CONSEQUENCES**
Peeters points out that people infected with group O virus can be resistant to one type of HIV medication. Understanding the genetic diversity of HIV, she says, "has implications for diagnosis, treatment, and also vaccine development."

—Kerry Grens



**ON TARGET:** A T cell expressing the protein Tim-3 (green) binds via T-cell receptors (red) to a cell targeted for destruction (blue).

IMMUNOLOGY

# Soluble Signal

**THE PAPER**
K.L. Clayton et al., "Soluble Tim-3 is shed from CD8+ T cells by the sheddase ADAM10, is increased in plasma during untreated HIV infection, and correlates with HIV disease progression," *J Virol*, doi:10.1128/JVI.00006-15, 2015.

**THE MOLECULE**
The Tim-3 protein on the surface of T cells is thought to dampen the immune response to prevent harmful overactivation. But in HIV infections, this protective mechanism is hijacked to exhaust T-cell function. Previous studies had found soluble Tim-3 (sTim-3) in the blood of cancer patients, but the role of circulating Tim-3 in HIV infections was not known.

**THE ASSOCIATION**
Kiera Clayton and her colleagues in Mario Ostrowski's lab at the University of Toronto, along with collaborators elsewhere, analyzed the blood of people infected with HIV and did indeed find sTim-3. Furthermore, sTim-3 levels correlated with patients' viral load, suggesting that the protein tracks with disease progression.

**T-CELL SURPRISE**
Despite the prevailing view of Tim-3's role in quieting immune activity, inhibiting T cells from shedding Tim-3 did not completely wipe out their function; in fact, some cells continued to release interferon. "Just because cells express Tim-3 doesn't necessarily mean that they're inactive as T cells," indicating a surprisingly complex function of the molecule, says Lawrence Kane of the University of Pittsburgh who was not involved in the study.

**DIAGNOSING FUNCTION**
The findings point to sTim-3 as a good biomarker for HIV and potentially for other chronic diseases such as cancer, says Kane. Further functional studies are necessary to determine whether disease progression relies on Tim-3, and, if so, perhaps could be slowed by lowering Tim-3 levels, says Clayton. "This does have implications in terms of treatment options."

—Jenny Rood

PIXABAY.COM; KIERA CLAYTON

# Putting It Together

Exploring viral replication pathways has led Carol Carter from the study of measles and reoviruses to the assembly and budding of newly minted HIV.

BY ANNA AZVOLINSKY

Carol Carter entered City College of New York wanting to major in biology and chemistry. But her freshman-year courses left her cold. "The classes were dull and uninspiring, and I was very discouraged," she recalls. Carter went to the professor who served as her freshman advisor and told him how much she hated the intro biology class. "The advisor leaned back in his chair with no change in facial expression and said, 'Wow, you're lucky.' This caught me completely off guard," she says. She remembers the advisor telling her, "You are lucky because you know what you want to do, so nothing is going to discourage you." Carter left the office even more perplexed than when she had entered, but after mulling over the encounter, finally understood the advisor's indirect message: a single experience or data point should not discourage someone with conviction.

> "I think that notion of finding a laboratory that becomes your own community is key, and that is the experience I had at both City College and Yale."

Carter persevered in her study of biology. She was encouraged to apply to graduate school by her undergraduate work-study employer, an ecology professor who became her mentor. "City College was and remains a strong teaching institution, and it prepared me well for graduate school."

As a graduate student at Yale University, Carter became fascinated with viruses. "Animal viruses were the new guys on the scene in the 1960s. Bacteria, phage, fungi, and parasites had held court in microbiology for a long time. It was a new field that attracted many young scientists." To Carter, animal virology was not only very exciting but provided opportunities to impact health and disease. She worked on measles virus and on reovirus, a related but low-virulence model for rotaviruses that cause diarrhea in infants and children, and then switched to studying HIV in the late '80s, a time when very little was known about the virus's biology. Early on, Carter identified virus-encoded targets for AIDS treatment. More recently, she has concentrated on host proteins necessary for HIV particle assembly, and is currently investigating how they work.

Here, Carter talks about pursuing biology despite a frightening experience with a salamander, the importance of inspiring mentors, and how Kaopectate—an over-the-counter medication to treat diarrhea—figured in her postdoctoral work.

## CARTER, CULTIVATED

**A mind for inventions.** In elementary school in Harlem, New York, despite an overwhelming lack of resources in the school system, Carter's teacher noticed her love of reading and managed to provide her with books. "He gave me this tattered brown book called *Inventions*. It described commonly used things people had invented, and I must have read that book a million times. Now it seems like such dry material for kids, but I found it so intriguing."

**Unfettered support.** "Neither of my parents went beyond the 7th grade, but they strongly believed that education was a good path, so they supported my sisters and me," Carter says. "What I mean by that is they created an environment of support for us. My mother would sit with us while we did homework, and if one of us wanted a cup of milk or tea, she would bring it to us. It was that kind of contribution."

**A tale of a tail.** Carter landed a work-study position at City College with James Organ, who studied salamander limb regeneration. In her first week in the lab, she was charged with cleaning the animals' cages and got a firsthand introduction to regeneration. "I'm taking the salamanders out and changing the straw in the cages and one of them runs away! So I chase the little guy around the lab and finally grab him by the tail. And, well, it's a salamander, so he drops his tail off and runs away. I start screaming hysterically. Jim comes running into the lab and finds me holding this little wiggling tail. And of course he starts laughing."

**Wonderful counselors.** Besides having a good sense of humor, Organ was also a great mentor, says Carter. "He knew how to guide students interested in science. He introduced me to other faculty in the biology department." That interaction with professors outside of the classroom was very important for Carter.

**Off to Yale.** "The expectation in my family was that when I graduated from college I would get a job. But I was encouraged to apply and attend graduate school by Organ," Carter recalls. "My mother was not that open to the idea, but then one of the schools that accepted me was Yale. She was not particularly sophisticated with respect to colleges and universities, but she knew that name and encouraged me to accept."

**Nurturing lab culture.** At Yale in 1968, Carter joined the laboratory of virologist and epidemiologist Francis L. Black, who

COURTESY OF CAROL CARTER/STONY BROOK UNIVERSITY

## CAROL CARTER

Professor, Department of Molecular Genetics and Microbiology
Adjunct Professor, Department of Physiology & Biophysics
Stony Brook University
Stony Brook, New York

### Greatest Hits

- Discovered that measles virus has a nuclear phase of replication
- Contributed to studies that established the subacute sclerosing panencephalitis agent as a variant of measles virus
- Demonstrated that oligo(A) in reovirions is not necessary for replication
- Among the first to demonstrate that self-assembly of the capsid protein of HIV could be executed in vitro using recombinant protein
- Contributed to defining the specificity of the HIV protease to better target the enzyme with small-molecule inhibitors
- Among the first to identify Tsg101 as a cellular protein that interacts with the HIV Gag precursor protein, pinpointed the Gag binding sequence, and showed that Tsg101 facilitates Gag release by a mechanism distinct from that used by related viruses
- Established that HIV recruits calcium signaling machinery to stabilize viral assembly platforms at the cell membrane

studied how measles virus spreads in human populations and how it replicates in cell culture. "He was also a fun advisor and really took his role as mentor seriously, taking me to meetings and introducing me to his colleagues." A collaborator of his in the department, epidemiologist Ann Schluederberg, was another valued counselor and an "inspiring and supportive person with whom I could discuss my experimental results and ideas. Ann was never discouraging. If she didn't like my idea, she'd say, 'Why don't you think about that and let's pick it up next week,' which I learned was a cue that maybe the idea was not so great!"

Years later, Carter recalls that Shirley Kenny, the first woman president of Stony Brook University in New York, encouraged incoming undergraduates to find "homes," by which she meant laboratories, where they could build relationships and get to know the people doing research. "I think that notion of finding a laboratory that becomes your own community is key, and that is the experience I had at both City College and Yale."

**Virus gone haywire.** In the early 1970s, when Carter was working towards a PhD, measles was the second most common cause of childhood mortality worldwide, responsible for about a million deaths every year. It was thought that measles only replicated in the cytoplasm, but Carter's thesis work showed that the virus's RNA genome was also present in the host cell's nucleus, providing evidence that measles has a nuclear phase of replication. She also found that measles strains recovered from patients who had a persistent infection in the central nervous system (CNS) were distinct from those strains responsible for acute measles infections. This CNS infection, called subacute sclerosing panencephalitis (SSPE), is a potentially deadly inflammation of the brain occurring in about one in every 10,000 measles cases. Others later discovered that the virus is able to infect the CNS when mutations result in a defective viral protein.

## CARTER CARVES HER PATH

**A serendipitous meeting.** Carter met Aaron Shatkin, a virologist at the Roche Institute of Molecular Biology who later discovered the 5' cap on reovirus messenger RNA (mRNA) molecules, on the way to a 1972 Gordon conference on animal viruses in Tilton, New Hampshire. "The airline lost my luggage, so I had no toothbrush or comb. Aaron saw me looking distressed and offered to drive me to the nearest town to get what I needed. He was very kind to me at the meeting and at the end, offered me a postdoctoral fellowship in his lab." Carter's work on reovirus

showed that, unlike in other viruses, reovirus-encoded oligo(A)s are not essential for infectivity.

**A sense of humor.** Shatkin's laboratory was having trouble growing high-enough titers of reovirus. Carter suggested a trick she had used in graduate school to get measles virus to grow well in tissue culture—Kaopectate. "I suggested this to Aaron and it turned out to work beautifully with reovirus. A few years later, Aaron was giving a seminar and told the audience, 'Let me tell you about a really shitty experiment my postdoc did,' and then proceeded to describe the Kaopectate experiment," Carter recalls, laughing.

**A new opportunity.** In 1975, Carter joined the faculty of the Department of Genetics and Microbiology at Stony Brook University as an assistant professor, and she has been there ever since. "The chair of the department who recruited me, Joseph Kates (the discoverer of polyA on mRNA), was the youngest department chair at Stony Brook. What attracted me to Stony Brook was how unconventional Joe was. He had very nontraditional ideas, and he attracted faculty who were interactive, collaborative, and diverse."

**A fresh start.** NIH funding for reovirus research began to dwindle in the 1980s because illness caused by reovirus was not a major problem in the U.S. At the same time, "HIV emerged in a very dramatic way in this country," says Carter. Her transition to studying the virus that causes AIDS arose from a collaboration with Eckard Wimmer, who worked on poliovirus across the hall. Carter and Wimmer recognized certain similar strategies used by poliovirus and HIV: to make infectious particles, both form a precursor polyprotein that must be cleaved to carry out critical structural and enzymatic functions. They applied for and received a grant to use poliovirus as a model to begin to ask questions about HIV.

**HIV drug targets.** In the late 1980s, little was known about the function of HIV-encoded proteins, although it had been shown that the virus manufactures a protease related to one that causes hypertension. This enabled Carter to collaborate with several pharmaceutical companies seeking to identify AIDS therapies and to develop HIV-specific diagnostics, efforts that began to bear fruit in the early '90s. Carter's grad students Kathy Partin and Gabriele Zybarth and Wimmer's postdoc Hans-Georg Kräusslich helped define the specificity of the HIV protease to better target the enzyme with small-molecule inhibitors. Carter's laboratory also studied the virus's capsid protein, p24, as a potential drug target. Lorna Ehrlich, a research associate in the lab, demonstrated self-assembly of the capsid protein in vitro using recombinant protein. "There are still no FDA-approved drugs against the capsid, but some are now in development," Carter says.

**Hijacking cellular machinery.** In 2001, Carter's grad student Beth Agresta identified several cellular proteins, including cyclophillin A and Tsg101, the product of *tumor susceptibility gene 101*, that interact with HIV's Gag—the major viral precursor polyprotein necessary and sufficient for viral assembly. Along with postdoc Fadila Bouamr and undergrad Traci LaGrassa, grad student Lynn VerPlank identified the sequence in Gag used to recruit Tsg101. "Tsg101 is important for sending proteins the cell no longer wants to the cell's garbage pail to be degraded," explains Carter. "But HIV uses Tsg101 to help it escape from the plasma membrane." Grad student Jay Goff showed that blocking Tsg101 prevents HIV particles from budding. Later, in 2005 and 2008, Gisselle Medina from the lab provided evidence that Tsg101 directs particle release through a pathway that is distinct from those used by related retroviruses. Recently, Carter's laboratory found that Tsg101 enables HIV to recruit calcium signaling machinery to help stabilize the viral assembly platforms at the cell's plasma membrane. "We think that this ability to use calcium signaling is the means by which the virus is able to maintain the Tsg101 ESCRT machinery at the budding site, and this may help us in trying to target Tsg101 effectively," Carter says.

## CARTER COMMUNICATES

**HIV drugs.** "Drugs targeting the HIV protease changed the complexion of AIDS treatment in the early 1990s when the major therapy was AZT, which targeted the virus's reverse transcriptase," says Carter. "AZT was a really tough drug for many people to tolerate. Once the FDA approved these newer inhibitors and they were combined with anti–reverse transcriptase drugs, one really began to see an impact on the disease."

**Fast pace.** "The field is moving fast, but still, at this stage, every drug targeting a viral-encoded gene product requires physician monitoring, because resistant mutants emerge," Carter says. "On the one hand, we are very fortunate that the arsenal is strong enough to use these drugs for both treatment and prevention. But drug development is still critical until we have a drug the virus can't evade, or a vaccine."

**Re-education.** "The number of new infections each year in the U.S. has not declined. In the beginning years of the epidemic, there were many young people with many, many sexual partners," Carter says. "But as the evidence accrued that this was a risk factor in HIV transmission, there was an impressive sobering that shrank this high-risk population. Now, we are trending back, because people forget what constitutes risk behavior and the disease is not in the public eye. Continuous re-education is needed to inform and to remind."

**Beyond HIV.** "Tsg101 is critical for budding, not only of HIV but other viruses as well." Coming full circle, Carter's laboratory, in collaboration with Jon Leis, has identified small molecules that target Tsg101 and other budding factors. "If we can get some of these compounds to work, we may be able to target other diseases as well, including Ebola," she says. ■

# Filippos Porichis: Immunoregulator

Principal Investigator, Ragon Institute of MGH, MIT, and Harvard. Age: 33

BY KERRY GRENS

Many of the friends Filippos Porichis grew up with on the small Greek island of Limnos followed tradition and became fishermen. But his dream was to become a physician. Porichis attended the University of Portsmouth in England to study biomedicine, where immunology pulled him in and never let him go. "I was more interested in trying to understand why diseases are happening and how the immune system fails . . . than trying to apply clinical medicine."

He stayed at Portsmouth for a master's degree before returning to Greece to earn a PhD from the University of Crete and the Institute for Molecular Biology and Biotechnology. There, Porichis focused on HIV pathogenesis—in particular, how the virus infects macrophages, which go on to regulate T-cell function.

Following his PhD, Porichis spent a compulsory year in the Greek army, which gave him time to think about his future career direction. "I realized I wanted to work more closely on HIV and go somewhere I could be trained with the best techniques and the best people." His resolution brought him to the Ragon Institute of Massachusetts General Hospital, MIT, and Harvard, where he did a postdoc in the lab of immunologist Daniel Kaufmann. Porichis launched into a series of studies on the dysfunction of T cells infected by HIV. In a 2010 paper, he and his colleagues showed that PD-1, a receptor on T cells, upregulates a transcription factor whose activity wipes out the ability of $CD8^+$ T cells to kill infected cells.[1]

He then turned to PD-1's effects on $CD4^+$ T cells, which Porichis says had received less attention than HIV-specific $CD8^+$ T cells. Kaufmann, now based at the University of Montreal, says Porichis was especially skilled technically, and the PD-1 experiments required creating novel assays to measure the effects of immunoregulatory networks on T-cell function. "Filippos was very successful," he says.

Using patients' blood samples, the team found that blocking the PD-1 pathway boosted $CD4^+$ T-cell function, even among people who had undetectable viral loads.[2] "This was proof of principle that immunotherapeutic interventions could complement antiretroviral treatments," Porichis says.

To probe deeper into PD-1's ability to so profoundly mess up T cells, Porichis and colleagues looked at the epigenetic regulation of the gene *PD-1*. It turns out that in HIV-positive people with low viral loads, the promoter region of the gene remains unmethylated—in other words, the switch is turned on even when the virus stops actively replicating.[3]

In 2013, Porichis started his own lab at the Ragon Institute. He's now focused on how healthy T cells function, with the aim of identifying ways to help combat viral infection. Most recently, he developed an assay to detect proteins, microRNAs, and mRNAs at the single-cell scale.[4] "It takes immunology to a new level," he says.

Porichis also leads the institute's international programs. One of his main responsibilities is to introduce students at Harvard Medical School to unmet medical needs in Africa. Ragon Director Bruce Walker says Porichis campaigned to have South African students involved as well, bringing them to the U.S. and having them accompany the Harvard group on a trip to Africa. "Filippos's interest is to build scientific capacity in Africa," Walker says, "and I think this really contributes to that." ■

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# Show Me Your Moves

Updated classics and new techniques help microbiologists get up close and quantitative.

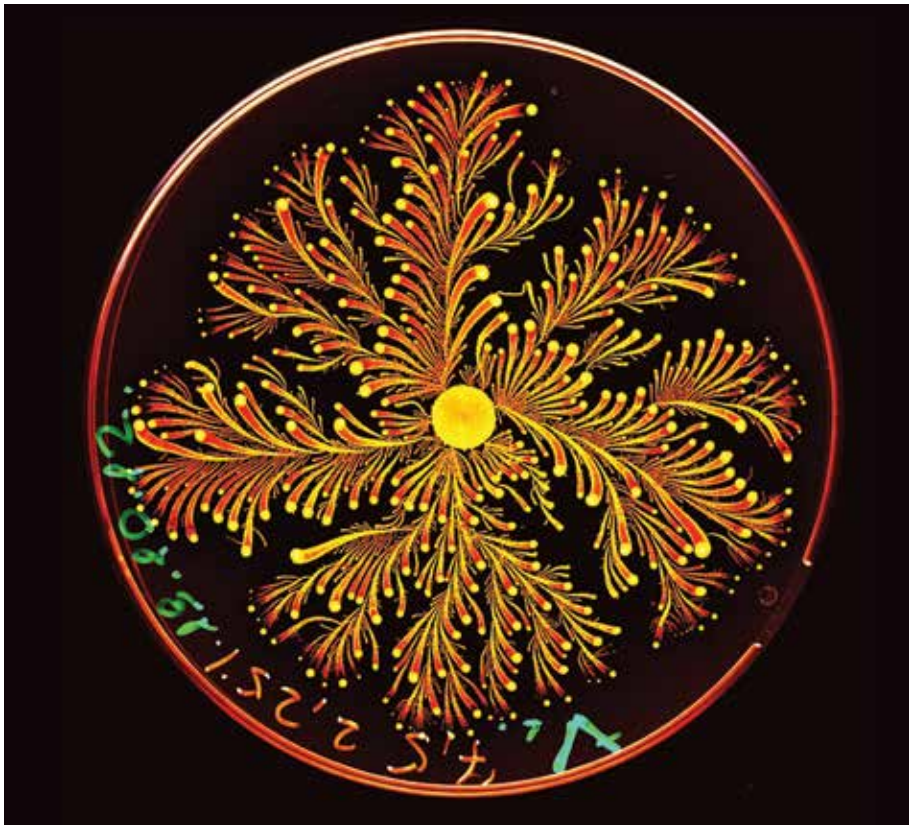
BY MARISSA FESSENDEN

Ever since Antonie van Leeuwenhoek espied the cavorting, swiftly swimming tiny critters he called *animalcules* through a small sphere of glass held in a metal frame, microscopes have figured into microbiological advances.

The stunning diversity of microbes, whether harvested from the human gut or scraped from the ocean floor, has increasingly led researchers to explore microbial behavior. As research entered the age of DNA, microscopes fell out of favor, and gaps in understanding the twitching, swimming, or creeping movements of microbes individually and as a colony have persisted.

Studying bacterial behavior requires techniques to view, track, and analyze these organisms in motion. Today, this involves new tools, such as genetically encoded fluorescent reporters, and improvements on old ones, such as quantitative methods for analyzing the complex swirls and spirals of bacterial colonies growing on agar plates. Even microscopes have made a comeback over the past two decades, thanks to the advent of small, relatively inexpensive cameras and increasingly sophisticated image-analysis programs, explains biologist Nicolas Biais of Brooklyn College.

*The Scientist* sought out some creative solutions for studying microbes on the move, en masse or one by one.



TRAVELING TOGETHER: A *Paenibacillus vortex* colony, 8 cm in diameter. The bright dots are dense groups of bacteria, termed vortices, that swarm collectively around a common center. As the cells replicate, the vortex expands and moves outward as a unit, leaving behind a trail of older, nonreplicating cells, which compose the branches. The leading vortices send signals to prompt the cells in the branches to generate new, fast moving vortices that become new leaders. (Color added; yellow indicates high cell density, red indicates low cell density.)

COURTESY OF ESHEL BEN-JACOB/INA BRAINIS

## ANALYZING BACTERIAL SWARM PATTERNS

RESEARCHERS: Eshel Ben-Jacob, professor of physics, Tel Aviv University, and adjunct professor of biochemistry and cell biology, Rice University; Colin Ingham, chief scientific officer, MicroDish, Utrecht, Netherlands

CHALLENGE: Early microbiologists quickly recognized that the swirls and streaks in their petri dishes differed among bacterial strains. The simplest interactions between strains were easy to decode: when competing strains grown in the same dish meet, for example, the boundary region—called the Dienes line—is starkly visible. But the human eye tends to misinterpret the patterns in more complex dynamics, says Ingham.

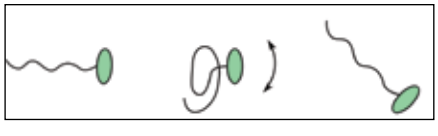
SOLUTION: For Ingham, formerly a senior scientist at Wageningen University, describing bacterial colony patterns quantitatively meant bringing in mathematicians and physicists. He teamed up with Ben-Jacob to study the swarming behavior of a pattern-forming bacterium that Ben-Jacob's lab group had discovered in the 1990s called *Paenibacillus vortex*. Together they returned to more traditional methods of observing patterns at the colony level through a microscope. Bacte-

rial swarming has practical applications in health-care settings, where you'd want to prevent it from happening. The coordinated, almost intelligent movement of many agents that aren't considered intelligent can also inspire cybernetics. (See "Crowd Control," *The Scientist*, July 2013.)

Under controlled growing conditions, the researchers subject their bacterial colonies to different stressors, such as nutrient deprivation or changes in humidity. Then they stain and image the colonies to figure out how those challenges affect bacterial motility and pattern formation. "These are very straightforward techniques," says Ben-Jacob. "But you need to look at the population level to see how [the microbes] talk to each other."

Through their collaboration, Ingham and Ben-Jacob uncovered traffic-like organization patterns in the swarms of *P. vortex* snaking across soft agar. On harder agar surfaces, the bacteria form complex colonies studded with vortices from which the colonies expand—features that inspired *P. vortex*'s name. By tracking mutant cells across the frames of time-lapse photos and measuring values such as the bend angle of the snake-like arms and the speed of different subpopulations in the colony, the researchers came up with the first quantitative description of swarming dynamics by the bacterium (*BMC Microbiol*, 8:36, 2008).

Further work elucidated the simple rules that can give rise to "decisions" about the direction of colony movement (*PLOS Comput Biol*, 7: e1002177, 2011), and revealed *P. vortex*'s apparent cooperation with the nonmotile fungus *Aspergillus fumigatus*. The moving bacteria carried fungal spores along with the swarm and crossed air gaps in the agar, which normally form an impediment to bacterial movement, over bridges made from fungal mycelia (*PNAS*, 108:19731-36, 2011).



MODES OF MOTION: *Rhodobacter sphaeroides* swims by rotating its single flagellum and tumbles by stopping it (center), causing the flagellum to relax.

COURTESY OF GABRIEL ROSSER

DIY: Ingham highly recommends ImageJ, a public-domain image-processing and analysis program developed at the National Institutes of Health (NIH). The program can be run online or downloaded and includes a wide variety of plug-ins for many applications. Microbiologists use it to analyze shapes, scan frame by frame through a video for movement, and more. The user base is active and committed. Questions and problems can be posted online at the NIH site and "the chances of finding someone who can answer are pretty good," Ingham says.

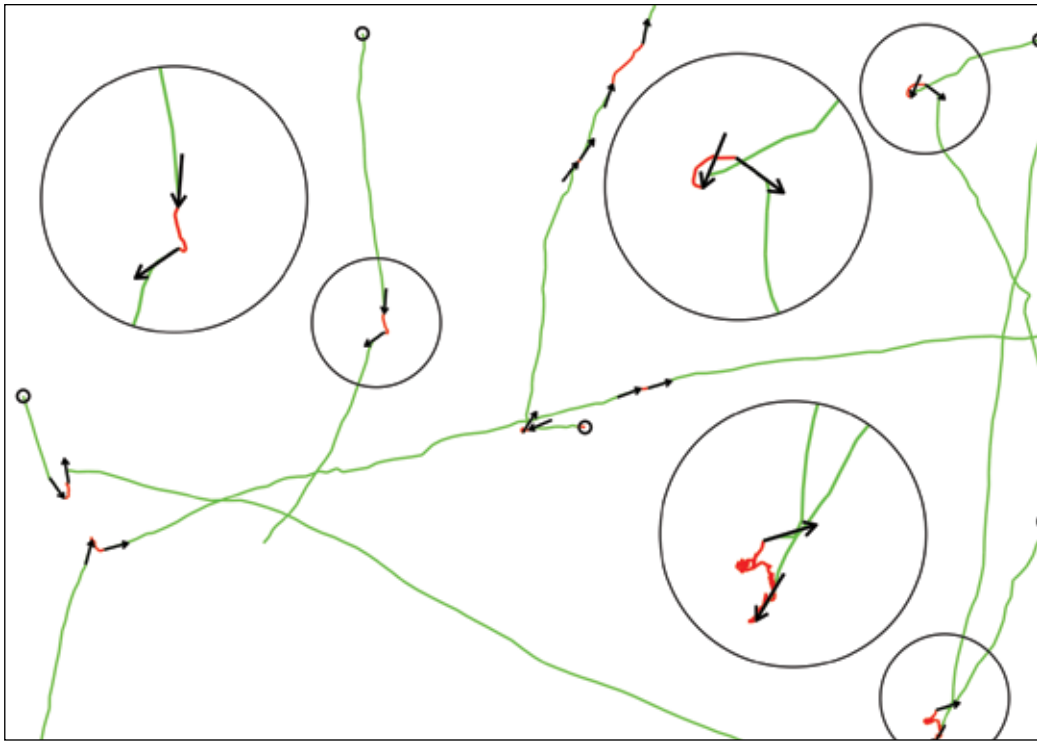
## TRACKING MANY BACTERIA AT ONCE

RESEARCHERS: Alexander Fletcher, applied mathematician, Oxford University; Gabriel Rosser, research associate in civil, environmental, and geomatic engineering, University College London

CHALLENGE: Typically, researchers laboriously track the movements of individual bacteria across their field of view. Enough single tracks can yield general conclusions about the species' movements. However, advances in imaging and computing now allow the capture and analysis of multiple tracks at a time. After a colleague generated a way to gather hundreds of bacterial tracks in one go—based on a program intended to track submarine movements—Rosser wondered what could be done with all the data. "I knew he had a valuable resource," he says. "But he wasn't really sure where to begin."

SOLUTION: Instead of choosing a subset of tracks to analyze, Rosser, then a PhD student in Fletcher's lab, developed algorithms that would take all the tracks, throw out ones too messy or too improbable to be real, and analyze the remainder. "The minute you choose a bacterium, you have to

MOVES IN MOTION: In this selection of individual *R. sphaeroides* tracks, green indicates a running phase, red indicates a stopping phase, small circles mark the starting position of the track, and pairs of arrows show the direction of travel of the bacterium immediately prior to and after a stop. Larger circles are expanded regions of track demarcated in the medium-size circles.

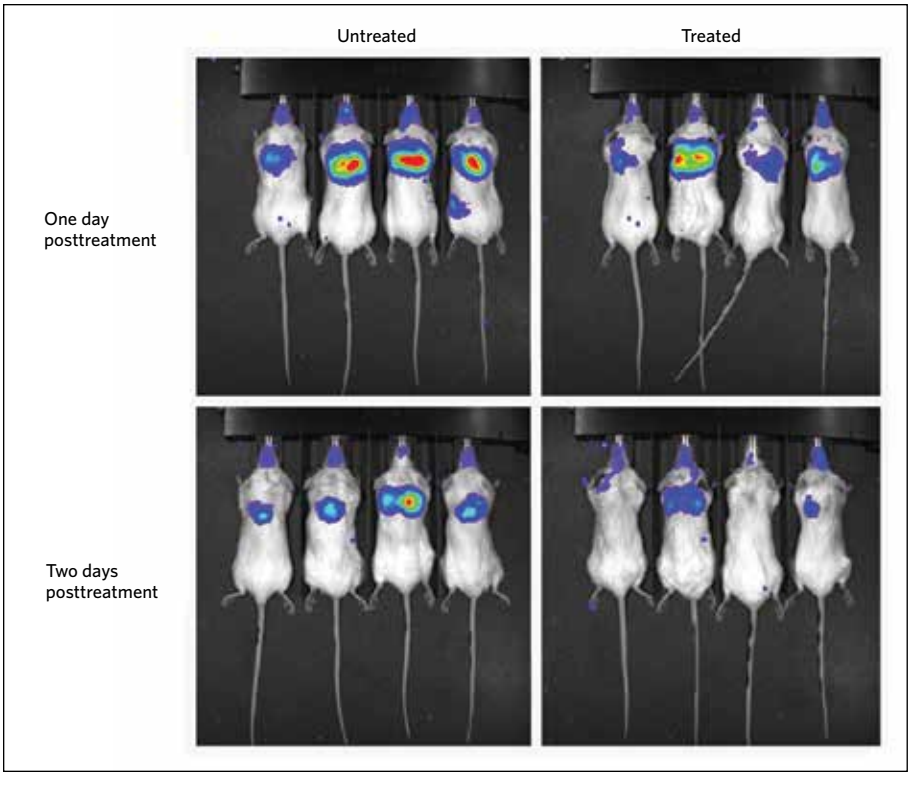


ask what kind of bias are you introducing," Fletcher says. You can choose more tracks that are 'good' tracks—straighter, longer and easier to analyze—than shorter, more tortuous tracks that nevertheless are part of the average movement of the population. In their method, the computer does the choosing.

They studied *Rhodobacter sphaeroides,* free-swimming, rod-shaped bacteria that use a single flagellum to move about, as do many liquid-dwelling microbes. Because the organisms are so tiny, the random Brownian motion of molecules surrounding them keeps them from achieving straight trajectories. Instead, they employ a strategy dubbed "run-and-tumble," where a quick spin or flick of the flagellum sends the bacterium on a short, straight run before it comes to a halt. A tumble, presumably influenced only by that Brownian jostle, sends the bacterium spinning before it can run again. However, by mathematically extracting out the runs and tumbles of many bacterial tracks, Rosser and his colleagues determined that the tumble phase wasn't random, as previously thought, but somehow mediated by the bacterium (*J R Soc Interface,* 11:20140320, 2014). "They change angle too quickly for it to be a passive process," says Rosser.

The breakthrough came after computer algorithms processed data from two mutant strains. One couldn't run at all and tumbled in place, buffeted by its environment. The other ran and never stopped. Together the two mutants modeled the two phases of the wild-type bacterium and a way to analyze the tracks.

**DIY:** The analysis method Rosser worked on, along with instructions for its use, is freely available for download in their report's supplementary information (*PLOS Comput Biol*, 9: e1003276, 2013). Bacterial tracks from typically used image-capture programs can be fed into the tools. Some of the apps Rosser wrote for cleaning up the data might need a few tweaks depending on the bacterial species, he says, "but the analysis should be fairly generic."




## DETECTING SMALL NUMBERS OF BACTERIA DURING INFECTIONS

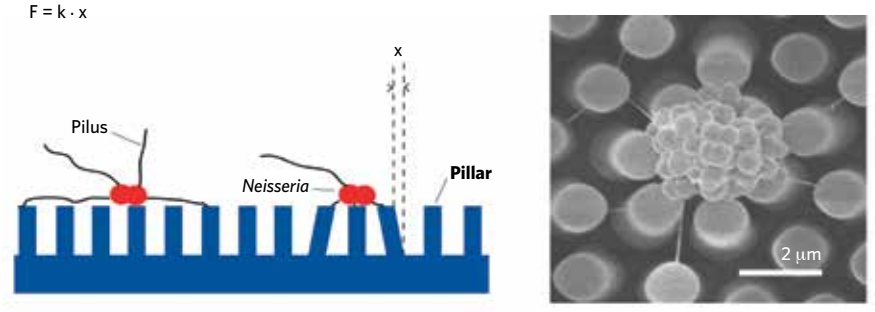
**RESEARCHER:** Jeffery Cirillo, professor of microbial pathogenesis and immunology, Texas A&M Health Science Center

**CHALLENGE:** The slow initial growth rate of many pathogens—particularly tuberculosis-causing mycobacteria—stymies efforts to understand how they infect their hosts. Researchers are still unsure exactly where *Mycobacterium tuberculosis* gains its foothold in the human body—the alveoli or the nasal pharynx—or what cues the pathogen to spread throughout the body. "Being able to track in vivo is a huge step forward for us," says Cirillo.

**SOLUTION:** To pinpoint such low numbers and selectively signal the presence of tuberculosis in samples that might be swarming with other bacteria, Cirillo's group developed a fluorescent reporter that only activates when the TB bacterium is near. Typically, organism-specific fluorescence reporter genes are engineered into the pathogen's genome for study in cell culture and in lab animals. However, this method promotes energy expenditure that wild-type bacteria don't employ in nature. "It may be a small difference, but it can impact the overall pathogenesis," Cirillo says, and thus experimental results. Instead, Cirillo's group embeds the fluorescent reporter within the chemical structure of nutrients the bacteria like to consume. The reporter-nutrient combination can be mixed into cell culture plates to identify clinical samples, or administered to lab animals, to help track moving infections. When the bacteria secrete a digestive enzyme, called BlaC, to break down those nutrients, the enzyme also cleaves

**LIGHT UP:** To learn how bacterial infections grow and spread from the lungs, Jeffery Cirillo and his colleagues use mycobacteria engineered to glow with the aide of luminescent molecules originally found in click beetles. Here, antibiotics quench infection and therefore the light readouts in treated mice.

M. CHANG ET AL., *PLOS ONE*, DOI:10.1371/JOURNAL.PONE.0108341, 2014.




**SWAY TO THE SIDE:** To measure the forces bacteria exert on their environment, Nicolas Biais adds a suspension of *Neisseria gonorrhoeae* and starts recording a digital movie. The bacteria attach to the microscopic pillars using hairlike projections called pili. By focusing on the tip of the pillars and how far they bend (x), he can measure the strength of the microbes' pull (F) after taking into account the pillars' springiness (deduced in a calibration step and denoted by k). A scanning electron micrograph shows a tiny colony of *N. gonorrhoeae* pulling on the pillars with their pili.

the reporter in such a way that a fluorescing molecule is released and starts glowing green (*Nat Chem*, 4:802-09, 2012).

The system can detect as few as 10 bacteria in a sample of human sputum (*Angew Chem Int Ed Engl*, 53:9360-64, 2014). Cirillo is also working on another system that uses bioluminescent molecules from the firefly and click beetle to track infections in living animals (*PLOS ONE*, 9:e108341, 2014). After tracking which organ is infected, the researchers can watch the infection grow and spread, Cirillo says.

DIY: The reporter system is based on genes that other groups discovered—Cirillo's team just made some tweaks. The plasmids for making the reporter-substrate are available in the open-source Addgene database.

## MEASURING FORCES EXERTED BY BACTERIA

RESEARCHER: Nicolas Biais, assistant professor of biology, Brooklyn College

CHALLENGE: The various structures that microbes use to get around are intricate molecular machines whose parts have been well characterized physically and genetically. But how different bacteria use these structures to propel themselves requires measurement of forces at microscopic scales.

Flagella in swimming bacteria have been well studied, but "we don't know squat about how [bacteria] move on surfaces," Biais says. His group studies long structures that bacteria can extend and retract called Type IV pili, which can be found on many infectious bacteria including *Vibrio cholerae* and *Neisseria gonorrhoeae*. Biais calls them "small-scale spidermen," because these microbes use their pili like grappling hooks to pull themselves along surfaces, interact with other bacteria, and attach to the epithelial cells of organisms they infect.

SOLUTION: Biais's lab group uses three methods for manipulating bacteria. All three involve giving the bacterium—usually a specimen from the team's model system of choice, *N. gonorrhoeae*—something with which to interact.

Optical tweezers produce a gradient of electromagnetic energy with the help of a highly focused laser beam. That energy can trap small objects and manipulate them as if using microscopic tweezers. Biais uses the tweezers to offer protein-coated beads to bacteria, which grab on and pull. By measuring a bead's displacement, the researchers can determine the force that the bacterium exerts. A camera mounted on a microscope captures the images for analysis in a program such as ImageJ or MATLAB.

However, the light of the laser produces heat that can change bacterial behavior. For a less disruptive manipulation, Biais builds a microscopic field of polymer pillars, using lithography methods similar to those that mold computer chips. The bacteria can move across the micropillars, like Spiderman across a cityscape, and Biais measures the displacement of each super-bendable "skyscraper."

Magnetic tweezers are cheaper, easier to use than their optical counterparts, and don't heat up cells. They work similarly, holding a magnetic bead in place to allow researchers to measure its displacement to determine force. However, these tweezers can't move as nimbly as the optical ones and are mostly useful for applying known forces and calibrating.

Using a combination of these tools, Biais's group has studied the pili of the *Neisseria* genus. The researchers found that a single fiber just 6 nanometers in diameter but extending up to 20 micrometers in length can exert forces up to 100 piconewtons. Bundled together in groups of 8 to 10, pili can maintain a 1 nanonewton tug for an hour (*PLOS Biol*, 6:e87, 2008). That force is equivalent to 100,000 times the bacterium's weight. Because such disturbances on mammalian cells trigger a signaling cascade to protect the cell from infection, insights into bacterial-epithelial cell interactions could offer new targets for antibiotics to attack.

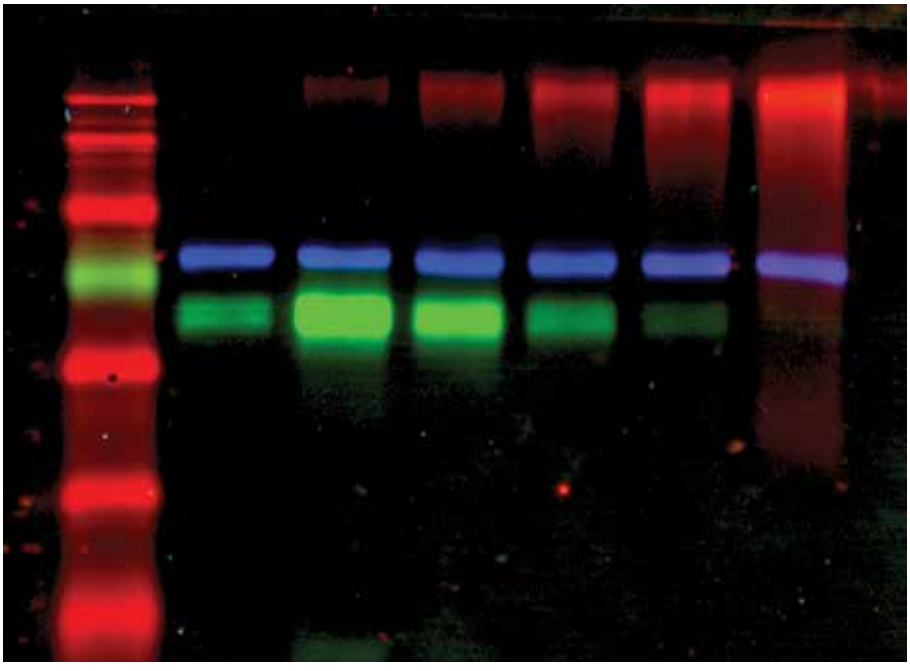
DIY: Lasers are expensive, but either optical tweezers or magnetic tweezers are needed to calibrate measurements on the micropillars. Biais has coauthored a book chapter that serves as a guide to using all three of these tools (*Methods Mol Biol*, 799:197-216, 2012), including fabrication methods for molding the micropillars. In another chapter, Biais delves into the details of magnetic tweezers (*Methods Cell Biol*, 83:473-493, 2007). However, given the range of forces bacteria can exert, tweaks may be needed depending on your organism. ■

N. BIAIS ET AL., *PLOS BIOL*, DOI:10.1371/JOURNAL.PBIO.0060087, 2008.

# All Is Not Quiet on the Western Front

A grab bag of advances is making Western blots faster, more sensitive, and more reliable.

BY KELLY RAE CHI



If you don't look too closely, Western blots are seemingly the same slog as when they were first described in 1979. You separate proteins by size (or charge) using gel electrophoresis, transfer them to a membrane, and probe the membrane using antibodies to your particular protein. Westerns have been a staple in protein research for many years, allowing researchers to identify, and sometimes semiquantify, proteins within tissues and cell cultures. It's easy to take them for granted. At the same time, the familiar blots are often the subject of scrutiny, fodder for fraud, and the reason behind some research retractions.

Western blotting is moving in the right direction, though. In recent years, new reagents and instruments have made Westerns more sensitive and have condensed some of the main steps, namely electrophoresis, blotting, and visualization. Although many researchers still use chemiluminescence detection and X-ray film, the introduction of digital fluorescence imaging has boosted the technique's sensitivity and reliability and pushed it into the "quantitative" rather than semiquantitative realm. New advances are also making it possible to study proteins in single cells, or to probe limited or precious samples—and to automate some parts of the process. And users are taking greater care in addressing the issue of reliable controls.

*The Scientist* spoke with researchers and companies working to make Westerns a little easier and more reliable. Here's what we found.

## SCALING IT DOWN

**BACKGROUND:** The past few years have seen great strides in measuring DNA and RNA in individual cells. By comparison, protein measurements have lagged behind. Poor antibody quality is usually the culprit, limiting how accurately researchers can measure protein levels in single cells using flow cytometry and immunocytochemistry. And separating the proteins, as done in Western blotting, is a must, but hasn't been possible to do using single cells until recently.

**APPROACH:** Amy Herr's bioengineering group at the University of California, Berkeley, has developed a microfluidic device they call the scWestern (for single-cell Western) that can perform Westerns on about 2,000 individual cells in less than four hours. The scWestern consists of thousands of microwells (20 µm wide by 30 µm deep) etched into a thin polyacrylamide gel coating atop a glass microscope slide.

The cells settle into individual microwells, where the scientists lyse them and apply an electric field. Lysates migrate through the walls of the microwell and into the photoactive gel, where the proteins separate by size. They are locked into the gel using ultraviolet light and probed with antibodies directly. Herr's group is able to analyze several proteins, in part by stripping the gels and applying different probes—up to 15 times.

Herr sees this technology as particularly important for measuring different protein isoforms and proteins that form complexes with other proteins or molecules.

**GETTING STARTED:** Although the technique is new, scientists outside of Herr's lab have, with some guidance, replicated the entire process, all the way from fabricating the devices to running the assays

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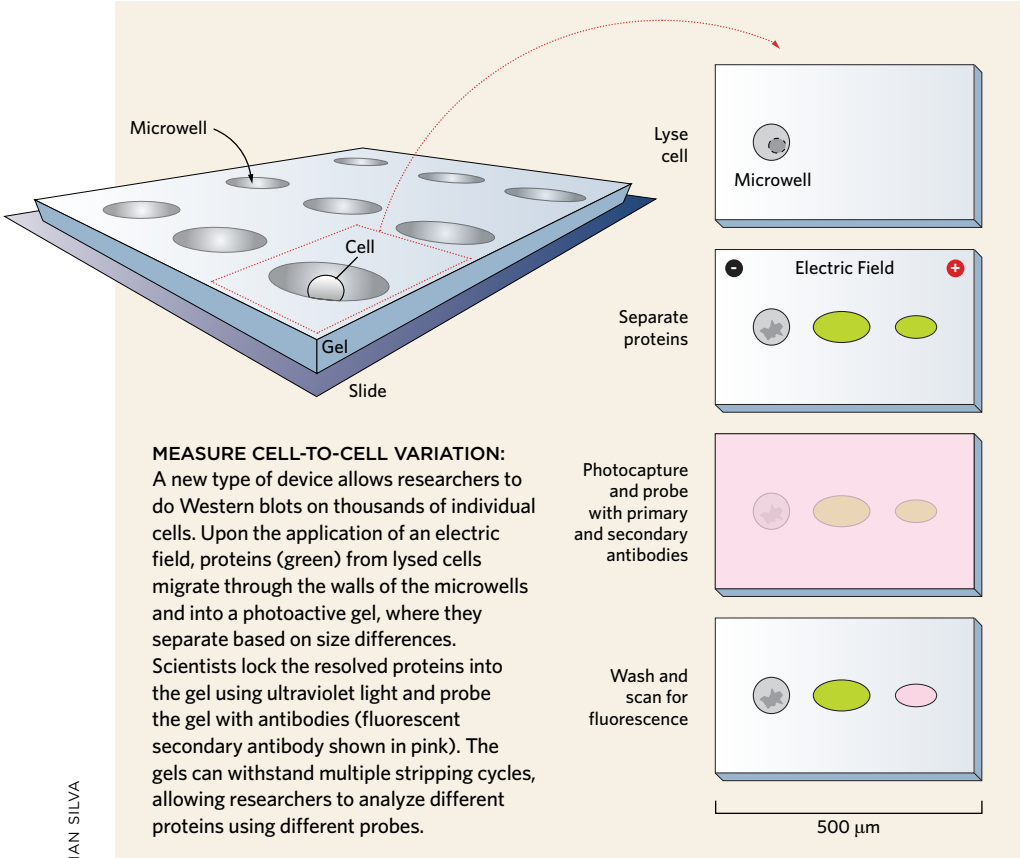



**MEASURE CELL-TO-CELL VARIATION:** A new type of device allows researchers to do Western blots on thousands of individual cells. Upon the application of an electric field, proteins (green) from lysed cells migrate through the walls of the microwells and into a photoactive gel, where they separate based on size differences. Scientists lock the resolved proteins into the gel using ultraviolet light and probe the gel with antibodies (fluorescent secondary antibody shown in pink). The gels can withstand multiple stripping cycles, allowing researchers to analyze different proteins using different probes.

(*Nat Methods*, 11:749-55, 2014). New graduate students in Herr's lab were able to get it down within a month, she says.

Herr's technology has led to the creation of the spinout Zephyrus Biosciences, which is developing a benchtop instrument that automates much of the handling. The company plans to launch by the end of next year.

**CONSIDERATIONS:** For now, the method has two limitations. One is that after the cells are lysed, proteins spill out of the microwells, resulting in losses of up to 40 percent. The second is that the separation resolution is subpar; researchers cannot separate proteins that are less than 50 percent different in weight. Herr's group is working on solving both of these problems. "I think we have some exciting results," she says.

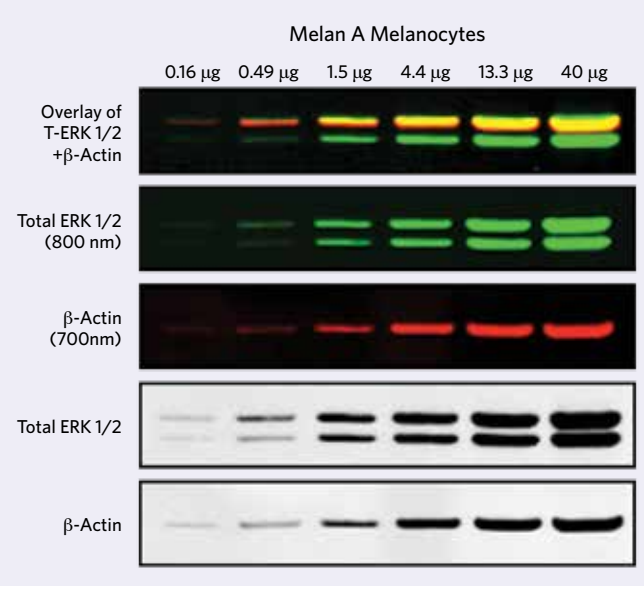
Microwestern arrays, including Herr's "µWesterns" (a precursor to her scWestern that uses larger wells), offer a gain in separation resolution for researchers who are willing to work with cell populations rather than single cells. The microwestern approach, which prints cell lysates on 96 tiny gel blocks arranged in the dimensions of a 96-well plate, is documented in a detailed series of YouTube videos (www.igsb.org/services/mwac-methodology) at the University of Chicago's Microwestern Array Core (MWAC) Facility website. The technique is able to resolve proteins that are at least 75 kDa different in size, if both proteins are more than 200 kDa. The technique requires the use of a piezoelectric dispenser, which can be quite pricey, says director Sam Bettis.

**COSTS:** The price of the benchtop instrument is TBD; fabricating your own scWestern costs a few thousand dollars if you purchase most materials fully prepared. The microwestern array alternative costs $1,800 per run (examining six cell lysates with 96 antibodies) through the University of Chicago's MWAC.

## SPEEDING IT UP

**BACKGROUND:** From start to finish, a traditional Western blot takes about two and a half days, which makes having to re-run it frustrating, especially if you have a lot of samples. "I do a lot of [Westerns], and trying to get through all of the samples was exhausting," says Jillian Silva, a postdoctoral researcher who studies melanoma signaling pathways in Martin McMahon's group at the University of California, San Francisco.

**APPROACHES:** Silva modified three steps in her Western protocol to cut her total run time down to one day. First,




**BOOST SPEED AND ACCURACY:** Two-color infrared detection is improving sensitivity, quality, and accuracy of Western blot data. Here, researchers used a LI-COR Odyssey (Classic) Infrared Digital Imaging System to detect and measure ERK1/2 and beta-actin in melanocytes simultaneously on the same membrane.

BASED ON *NAT METHODS*, 11:749-55, 2014, REDRAWN WITH PERMISSION FROM AMY HERR; COURTESY OF JILLIAN SILVA

she replaced her SDS-PAGE gels with a Bis-Tris gel system, which takes 35 minutes to run (as opposed to 1.5–2 hours for SDS-PAGE) and has the benefit of boosting sensitivity. Second, she purchased a rapid blotting machine (iBlot Dry Blotting System, Life Technologies), which transfers protein to the membrane in seven minutes. Using the old method, this step took one to two hours.

The third—and most important—step, Silva says, was to move from chemiluminescence to fluorescence detection. LI-COR's Odyssey series uses lasers that excite the membrane in two infrared wavelengths, 700 nm and 800 nm, allowing Silva to measure two different proteins on the same membrane. "That helped a lot because I could run all these different proteins at one time, whereas [before] I was running a gel for each of those proteins," she says.

**GETTING STARTED:** Silva has taught several other labs her protocol, which is available in the *Journal of Visualized Experiments* (e51149, 2014). She finds that people are initially hesitant to try fluorescence imaging, but once they do, they don't go back. Getting up and running takes some tweaking of, for example, blocking buffer amounts and antibody incubation times, but she says it's well worth it for the higher speed and sensitivity.

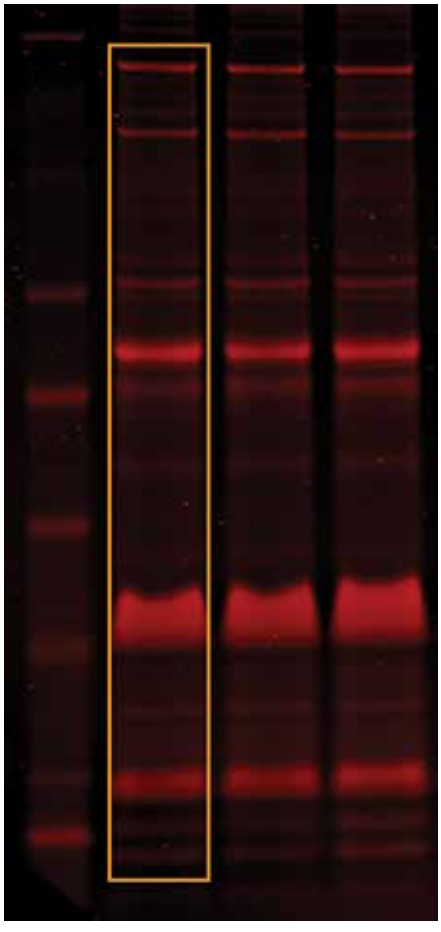
**COSTS:** $14.50 for a Bis-Tris pre-cast gel; $1,996 USD for the iBlot 2 Gel Transfer Device; $30–60K for a LI-COR Odyssey Infrared Imaging System. Prices vary by region.

**CONSIDERATIONS:** The time savings come with an added cost, however. Silva estimates about $20 extra per experiment for the Bis-Tris gel and iBlot consumables. But you can account for the time saved, either in personnel costs, animal maintenance costs, or both. Also, if you opt for fluorescence and digital imaging, you won't have to pay for chemical developer and fixer, vendor maintenance of film processing machines (about $324/year for a user in Silva's department), and, of course, film.

## TOTAL-PROTEIN CONTROL

**BACKGROUND:** To help quantify proteins of interest, researchers typically compare them to single proteins also present in the lysate, such as GADPH, β-actin, or tubulin, which are thought to occur at consistent levels across cells and tissues and over time.

However, recent evidence suggests that levels of these so-called loading controls can vary across disease states and in different places within the same tissue, and may skew results by up to 20 percent (*PLOS ONE*, 8:e72457, 2013). "Many of the proteins that people commonly use as loading controls . . . are not stable at all, and they actually change in a broad range of different neurodegenerative conditions," says Thomas Wishart, a research fellow at the University of Edinburgh's Roslin Institute in the U.K.



TAKE THE WHOLE LANE: Researchers are realizing that individual housekeeping proteins, typically used as controls because they were thought to be consistently expressed, can vary in their amounts across different tissues and disease states. Analysis of the total protein concentration within a sample (highlighted in the yellow box) is a better reference for quantification of proteins.

**APPROACH:** Wishart's group instead compares its proteins of interest to the total protein levels in the same lane, and has found that to be a more reliable method for normalization. That involves running two gels in parallel, and soaking one of them in Coomassie blue dye. Then, using digital imaging software, the researchers measure protein signal along the length of the entire lane.

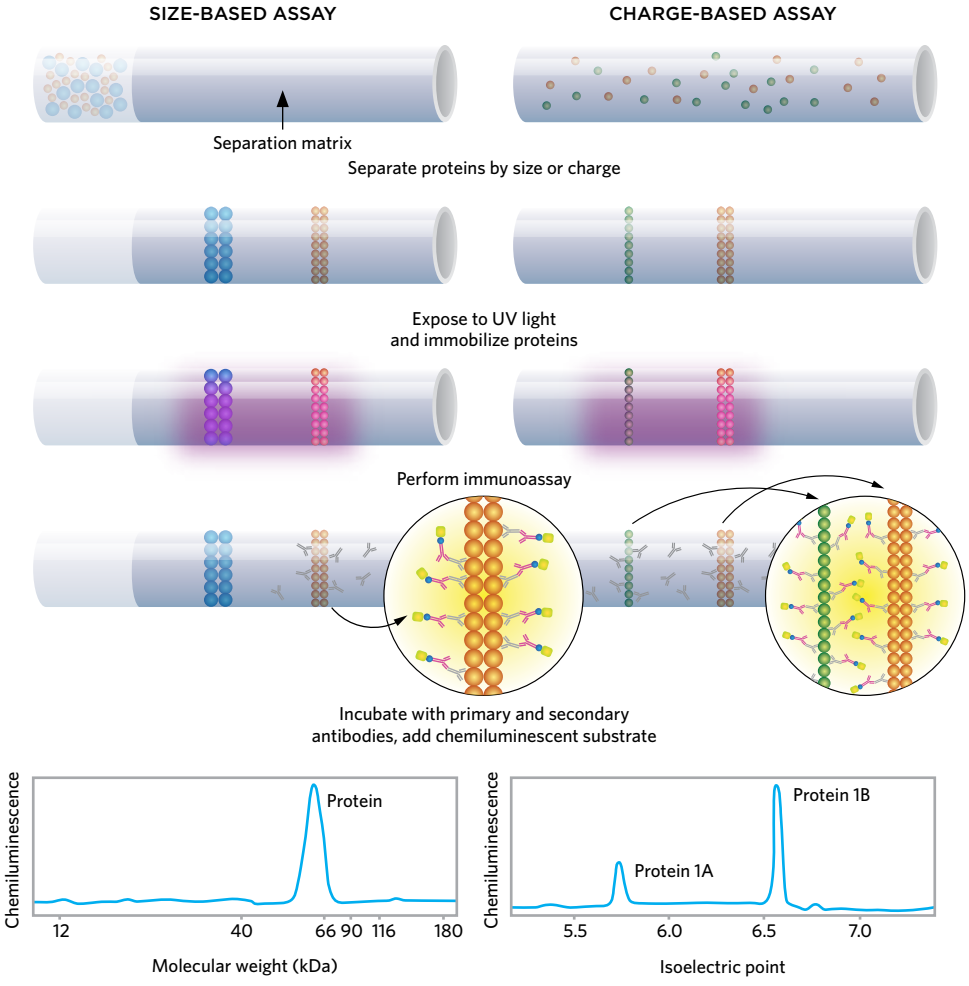
**GETTING STARTED:** Wishart's protocol, with troubleshooting tips, is available online (*J Vis Exp*, e52099, 2014). Having to load a second gel can introduce another potential source of error, but as long you can pipette single-digit µL volumes consistently, it shouldn't be a problem, Wishart says. It helps to cross-check your Coomassie results with protein concentration in a BCA or other protein assay.

Wishart's approach is one of many subtle variations on doing total-protein normalization. Bio-Rad, for example, makes "stain-free" gels that you can scan for total protein before transferring and probing for your protein of interest, though this approach requires special equipment for visualization.

**COSTS:** Wishart's protocol adds about 20 percent to your costs to run and stain that extra gel with Coomassie. "[But] realistically, you're probably saving money in the long run because you're doing it right from the start," he says.

**CONSIDERATIONS:** If you insist on using individual proteins as loading controls, look through published proteomic data sets (using mass spectrometry) for proteins whose expression levels don't seem

IMAGE COURTESY OF SAMANTHA EATON, THE WISHART LABORATORY, THE ROSLIN INSTITUTE, U.K.




REDRAWN FROM PROTEINSIMPLE.COM

**LOOK, NO HANDS:** ProteinSimple's Simple Western systems use matrix-filled capillaries to resolve proteins based on size, charge, or both. The automated system shines ultraviolet light on the capillaries to immobilize resolved proteins, probes with primary and labeled secondary antibodies, and then quantifies the resulting signals.

to vary and are therefore more likely to be reliable.

## GOING HANDS-FREE

**BACKGROUND:** The multiple steps of traditional Western blots have remained much the same since the technique's initial development. Although the steps may feel automatic to some scientists, the more handling and incubation steps there are, the more likely something will to go wrong. Researchers recognize the results as semiquantitative at best.

**APPROACH:** The San Jose, California, company ProteinSimple has come up with a different way of doing Western blots by using a matrix-filled capillary rather than a gel slab to separate proteins based on size, charge, or both. The subsequent steps of immobilizing the proteins, probing them with antibodies, and visualization are fully automated, making the firm's instruments the most automated of the Western blot systems available.

In 2011, the company launched Simon, the first in its line of Simple Western systems. The newest instrument, Wes, launched in 2014, is marketed for individual labs and analyzes up to 25 samples in less than three hours. Sally Sue and Peggy Sue are higher throughput, analyzing 96 samples per run with as little as 0.2 µg/µL of cell lysate.

"Besides improved reproducibility and consistency of the results, the biggest advantage, and this is especially significant for charge separation, is the ability to assay very quantity-limited samples," says Joanna Liliental, director of the Translational Applications Service Center at the Stanford School of Medicine, which uses a Peggy Sue and its charge-separating precursor NanoPro 1000. "For a charge-separation assay, depending on the abundance of target protein in the cell, it is possible to quantify signal from only 25 cells. You'd never be able to do that with a [standard] Western."

**COSTS:** Although prices vary by region, Wes is priced "similar to a high-end imaging system," says Simple Western product manager Patricia Piatti. Simple Western's consumables are more expensive than if you made everything from scratch for a traditional Western; Piatti equates the costs to those of an "average" conventional Western.

**GETTING STARTED:** More cores are adding Simple Western instruments. Some of them, such as Liliental's TASC, serve external users for at-cost pricing; it might make sense to consult with a core before considering the purchase of an instrument.

Of TASC's 70-some users, only a few are trained on the instrument; mastery requires a four hour training session and a couple of weeks of regular use to become comfortable manipulating the machine. The software can take a bit longer to learn, depending on how complex the experiment is, Liliental says. With these systems, most of the work falls into planning the experiments to get the most out of each run and interpreting the data, she adds.

**CONSIDERATIONS:** As with any Western blot method, the quality of your antibody can be a limiting factor. ProteinSimple and its sister company Bio-Techne are working to validate antibodies for use with Simple Western instruments. "This is an ongoing project, and we'll continue to add more certified antibodies to our already long list," Piatti says. ■

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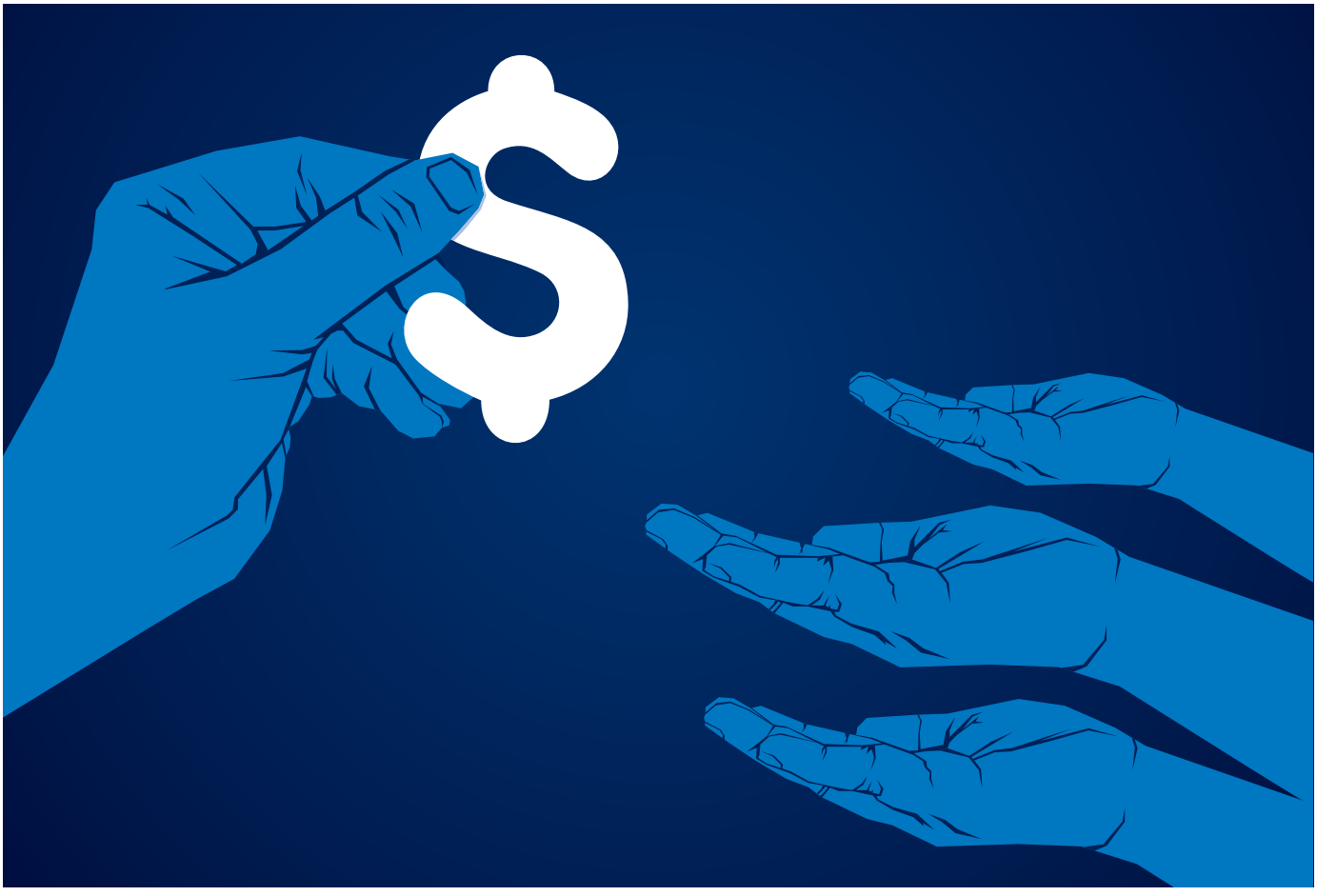
# Follow the Funding

In times of budget belt-tightening at the federal level, life-science researchers can keep their work supported through private sources.

BY BOB GRANT





A few years ago, David Sinclair's lab was slipping through his fingers. With grant money running dry and the outlook for overall federal research budgets bleak, the Harvard geneticist was losing lab members because he couldn't support them with funding from the National Institutes of Health (NIH), as he had done in years past. Sinclair says his 18-person-strong group dwindled to just four or five people. "And that was painful," he recalls. "I had to let people go for lack of money."

And Sinclair says he's not alone. "Even at a place like Harvard, I know [other] labs that have downsized dramatically and even closed down," he says. "So it's hit across the board."

In the face of such persistent financial stress, Sinclair was forced to reconsider his funding strategy. Rather than try to secure a few generous, but highly competitive, NIH grants, he began applying for diverse, smaller grants from private funding sources, such as foundations or corporate collaborations. "I didn't really have a global plan," Sinclair admits. "Really it's just trying to get by every day. You try to keep your lab going at the same level any way possible."

Many other scientists are similarly seeking private, rather than public, funding sources, as the federal budgetary sequestration extends into its second year and government research budgets show no signs of rebounding any time soon. Nir Barzilai, director of the Institute for Aging Research at Albert Einstein College of Medicine, used private money not only to fund his own lab, but to launch an entire research center. He and collaborators opened Einstein's Paul F. Glenn Center for the Biology of Human Aging in 2012 with the help of a $3 million grant from

the Glenn Foundation for Medical Research, and funding from private sources continues to fuel research at the center. "For me personally, the NIH hardships, I survived them," Barzilai says. "When I hear of any private source, it's immediately an e-mail to the people at the Center. . . . I probably have as much funding as I had before."

**The recent success of the biotech financial markets, with an unprecedented number of IPOs, has really brought a lot of money into the hands of younger companies that are focused on new biology, and are now valid sources of funding.**

—Michal Preminger,
Harvard University Office of Technology Development

*The Scientist* spoke to researchers using private funding to make ends meet and learned some of the tricks to finding and securing grant money even in these hard times.

## A funding foundation

Numerous charitable foundations award small or medium-size research grants, usually in the range of $100,000 to $300,000, to scientists working on biological phenomena relevant to particular diseases or disorders. Sinclair has been able to secure funding from several philanthropic organizations—including the Glenn Foundation for Medical Research, the Juvenile Diabetes Research Foundation, and the United Mitochondrial Disease Foundation—as well as grants from Harvard that are meant to accelerate commercialization, and even gifts from private families, for work on diseases that involve mitochondrial malfunction. A bevy of charitable grants like these, though individually not often as robust as R01s from the NIH, can sustain a lab full of grad students, postdocs, and researchers.

Evris Gavathiotis, a biochemist at Albert Einstein College of Medicine, has even used private foundation funding as a means to gather data that he can then use to write more-compelling NIH grant proposals. As a new faculty member in 2011, he knew he would need an R01 grant from the NIH to get his lab up and running. But his October 2012 proposal on a novel pharmacological strategy to induce apoptosis in cancer cells was rejected because experimental evidence supporting the approach was lacking. So Gavathiotis sought private funding to support the preliminary experiments. In 2013, he won a $200,000, two-year grant from the Sidney Kimmel Foundation to do in vivo toxicity experiments to show that his small-molecule approach to targeting PAX proteins was worthy of further investigation. When he resubmitted his R01 proposal later in 2013, it was accepted, and he was awarded the grant last year.

"We want to get NIH funding; it gives sustained funding to the lab," Gavathiotis says. "[But foundation grants] help you fill the gaps if you don't have an R01 or help you build up your research program to get to the point where you would be competitive for an R01."

Just after getting his R01 grant last summer, Gavathiotis got another charitable grant, this time from the Michael J. Fox Foundation, which injected $165,000 into a project to discover drugs for Parkinson's disease. Gavathiotis says that he plans on submitting proposals for at least two more R01s, but in the meantime, he will continue to apply for private grants as well. "It will give us some additional funding to finish these projects and get some more papers out," he says. "Eventually the R01s will come."

Relying on private funding to propel your research can, however, mean adjusting the goals of your work, notes Harvard's Sinclair. "We do have more of a translational focus in general," he says. "We're not as free to do what we want to do as we used to be."

Gavathiotis agrees. "There are not many foundations where you can get a grant on something where there's no application. That's how they work."

## Going industrial

Another source of private funding—one that necessitates even more of a translational approach—is the biotech and pharmaceutical industry. Sinclair's group is working with pharmaceutical company Novo Nordisk, for example, searching for therapeutic proteins to treat type 2 diabetes. That collaboration alone has provided two years of funding and supported one postdoc in his lab, says Sinclair, who adds that his lab and the company recently extended the project.

Rather than simply partner with industry, Barzilai decided to join it, cofounding a biotech company with Sinclair and others as a way to generate money for basic research. In 2011, Barzilai, Sinclair, and a couple of colleagues launched CohBar, which is focused on the discovery and development of novel mitochondrial-derived peptides to treat disease and extend healthy life span. According to Barzilai, CohBar has raised about $17 million in the past year—$5.7 million in private funding and $11.25 million through an initial public offering (IPO) on the Toronto Venture Exchange this January. He adds that the firm is even able to outsource some of its R&D to academic researchers.

Seeking out private funding has been the saving grace for many labs, especially Sinclair's. The total funding makeup of his lab, which now supports 22 people, is about 75 percent private and 25 percent federal. This includes just one NIH R01 grant, two projects that involve industry collaborations, funding from foundations, and a grant from Harvard's Blavatnik Biomedical Accelerator. In addition to keeping his lab open in the face of economic hardships on the federal level, Sinclair notes another benefit: "I've spent much less time in the last two years writing NIH grants." Plus, because his lab is primarily supported by private funding, Sinclair also spends much less time on administrative tasks, such as reporting and documentation, whereas NIH grants typically bring with them a heavy administrative burden.

## The benefits of privacy

Going the private-funding route may mean thinking of your research in more of a translational sense. But that doesn't have to be a negative, says Michal Preminger, executive director of the Harvard University Office of Technology Development. Preminger's office aids in translating Harvard research into commercial products. And that means she spends most of her time playing matchmaker between academic labs and biotech firms or investors interested in a particular field of research. Preminger says that her office has helped increase the amount of industry dollars going into Harvard labs four- or fivefold in the past three years.

Even without the federal research budget squeeze that academic scientists have witnessed in the past few years, Preminger says, life science was ripe for the flow of private funding into basic science labs via industry and venture capital investments. "The recent success of the biotech financial markets, with an unprecedented number of IPOs, has really brought a lot of money into the hands of younger companies that are focused on new biology, and are now valid sources of funding," even for academics, she says. "This is not really related to the need. It just happens to be the case that the life sciences have been able to deliver very interesting breakthroughs and innovation that have fueled the industry and raised the appetite for more."

In addition to attracting private funding, shifting the focus of a basic-research program to include thinking about applications of the biology under study can also reinvigorate the work itself, Preminger adds. "Sometimes federal funding makes [researchers] very comfortable in a basic-research domain, and so the ability to venture out of that and look at more translational work is, on the one hand, important if you're going to raise alternative sources of funding, but it also becomes a goal in its own right, once they're exposed."

Barzilai agrees. "[Starting CohBar] created the opportunity to take some of our most promising research and get it out there so it could do good for the public."

Viewed in that light, the federal funding crunch can actually be considered a positive motivating factor for many basic researchers, Preminger suggests. "In many ways this is the silver lining of the NIH budget crises: more interaction between the real world and the academic lab these days." ■

## GOING PRIVATE

Private funding opportunities are out there for the taking. You simply have to know where to look. Your own institution can be a big help in this regard, but you'll likely need to do some poking around on your own. Here are some resources that may help:

**Community of Science PIVOT funder database**
This website lists all sorts of funding opportunities for biomedical research, including state, federal, and international grants. But it is an especially good place to find private funding opportunities. You must be a member to use the service, but many universities have institutional memberships that may grant you access.

**State-based associations**
Groups such as MassBIO, the Washington Biotechnology and Biomedical Association, and NewYorkBIO often hold workshops and programs that are open to academics and can be great forums to make industry contacts and learn about specific topics, such as drug development, that could help you tailor your research program so it is more attractive to investors and commercial partners.

**Offices of sponsored research**
Most universities have offices that deal with providing faculty support and assistance in the administration of externally funded research. These offices will also send out announcements regarding foundation or philanthropic funding opportunities that are available. Getting on e-mail lists from these offices could prove invaluable.

**Scientific conferences**
Especially at big annual conferences, such as those hosted by the American Association for Cancer Research (AACR) or the Society for Neuroscience (SfN), potential funders are in attendance. Don't shy away from attending industry talks, stopping by commercial booths on the convention floor, or fielding questions from industry folks after presentations to make contact and inquire about funding or partnership opportunities.

**Philanthropic funding through your home institution**
Many universities have philanthropic grants that are available only to their own academic researchers. Your office of sponsored research, or equivalent service, should be helpful in pointing you in the right direction.

# Attacking AIDS on Many Fronts

A close cooperation between science, politics, and economics has helped to control one of history's most destructive epidemics.

BY PETER PIOT

The emergence of AIDS in the 1980s sparked one of the most disruptive events in modern times. The pandemic has not only killed millions worldwide, but it has also profoundly altered our approach to sexuality, doctor-patient relations, the influence of civil society in international relations, and northern hemisphere/southern hemisphere solidarity. It thrust health firmly to the fore of national and international politics, where it so rightly belongs.

In *AIDS: Between Science and Politics* I had the unique opportunity to reflect on my experience as a scientist-clinician, as founding executive director of the Joint United Nations Programme on HIV/AIDS (UNAIDS), and as an activist involved in the struggle against HIV/AIDS since the epidemic began. The book explains my fundamental belief that without political or economic relevance, science can bring little to people. Conversely, without scientific evidence and a respect for human rights, politics is ineffective and can even be harmful.

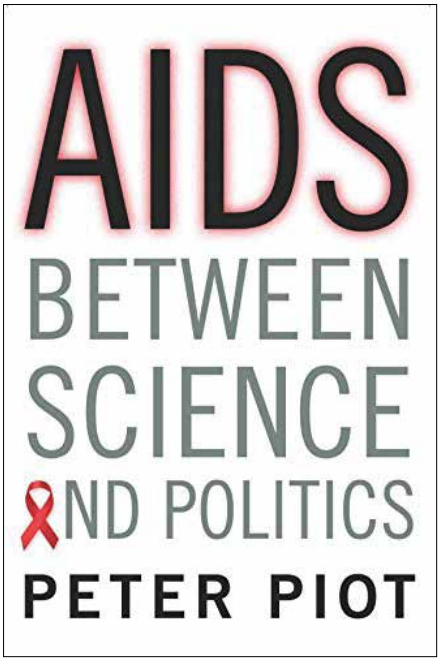
The first years of the AIDS epidemic were marked by the cruel stigmatization of those living with the disease (something that still exists today, albeit in sometimes subtler forms). But a powerful advocacy movement succeeded in slowly dispelling a fearful, reactionary, and often hateful perception of the disease. The AIDS movement changed the face of public health. It applied pressure to the scientific and political communities to do more, and it deepened our notions of security and human development as related to health.

Much of our progress is due to scientific discovery and treatment innovation, with the introduction in 1996 of highly active antiretrovirals (ARVs) being the most spectacular game changer. It is not an exaggeration to compare this breakthrough with the discovery of penicillin opening the antibiotic age. ARVs changed the lives of millions of people living with HIV, as well as how the world perceived AIDS and the epidemic. HIV infection was no longer a death sentence, and there was hope that one day the epidemic could be stopped.

In spite of these scientific advances, the overwhelming majority of patients in developing countries had no access to new medications. HIV continued to spread seemingly unhindered across the world, and patients continued to die. An absence of political will, denial by leaders in the most affected countries, and a lack of funding all fuelled the mounting death toll, especially in sub-Saharan Africa.

International solidarity for AIDS prevention and treatment came late, but the creation of novel international institutions such as UNAIDS and the Global Fund to Fight AIDS, Tuberculosis and Malaria became models for tackling other global issues. Support for the global HIV/AIDS response has greatly expanded to include bilateral aid, foundations, and nongovernmental organizations (NGOs), together with some inspiring examples of political leadership. For the first time in 30 years the epidemic has receded significantly on several continents. Since 2001, new HIV infections have fallen by 38 percent, and more than 14 million people are now receiving treatment.

Leaders and legislators have recognized the exceptional character of the epidemic, and the political sphere is now engaged at multiple levels. The considerable cost of AIDS, its economic impact in the most affected countries, and activist pressure have spurred governments to act.




*Columbia University Press, April 2015*

But we cannot claim victory over this disease; every day nearly 7,000 people are newly infected with HIV, and about 5,000 people die from AIDS-related illnesses. Relating the historic successes highlighted in my book should encourage a redoubled effort at AIDS prevention, treatment, and research, and a long-term increase in funding. Over the past three decades, the HIV/AIDS movement has been able to overcome what seemed to be insurmountable obstacles. It still has the energy and creativity to take on new challenges in the fight against what remains one of the greatest public-health tragedies of modern times. ■

*Peter Piot is the director of the London School of Hygiene & Tropical Medicine. He is a former Under-Secretary-General of the United Nations, the founding executive director of UNAIDS, codiscoverer of the Ebola virus, and former president of the International AIDS Society. Read an excerpt from* AIDS: Between Science and Politics *at www.the-scientist.com.*

**The Genealogy of a Gene: Patents, HIV/AIDS, and Race**
Myles W. Jackson
*The MIT Press, February 2015*

*CCR5* has been through a lot. Since scientists first sequenced it in the 1990s, the human nuclear gene—about 6,000 base pairs, located on chromosome 3—has gone from needle in a genomic haystack, to scientific curiosity, to patented product, to genetic blueprint for a key HIV coreceptor, to hotly researched potential target for AIDS drugs and vaccines. In *The Genealogy of a Gene*, New York University historian of science Myles Jackson exhaustively describes *CCR5*'s journey through wet labs, the US patent office, biopharmaceutical firms, popular consciousness, and beyond. "In short, the gene's genealogy is a complex and intertwined one," he writes, "including lineages of biocapitalism, the sciences of chemokines and HIV/AIDS, the Human Genome Project and the HapMap, intellectual property law, big and small pharma, personal genomics companies, and race."

Jackson stresses that his book is not a complete biography of *CCR5*, as the gene's story is still very much unfolding. Just last year, for example, researchers elucidated the structure of the complex that the CCR5 protein forms when CCL5, an inflammatory cytokine, binds to it, paving the way for designing novel CCR5-targeted AIDS drugs. Through the lens of a relatively short gene buried within the human genome, Myles dissects the scientific, industrial, and sociological forces that have shaped the genomic research enterprise over the past 20 years.

**On the Move: A Life**
Oliver Sacks
*Alfred A. Knopf, May 2015*


Renowned neurologist and prolific writer Oliver Sacks has always injected his own experiences and emotions into his gripping tales of disease and brain science. But now he has penned the most personal work of his long and distinguished career. In his autobiography, *On the Move*, Sacks leaves no stone unturned as he delves into all points—pleasurable, painful, and otherwise—of his life. From his boyhood fixation on motorcycles, to the clinical experiences with encephalitis lethargica that inspired his first major book, 1973's *Awakenings*, to love affairs, struggles with drug addiction, and his bond with his schizophrenic brother, Sacks offers an intimate look at the defining moments of his journey thus far.

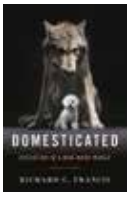
Sacks's autobiography comes at another crucial crossroads in the 81-year-old author's life. He revealed in a February *New York Times* opinion piece that the cancer detected in his eye nine years ago has metastasized to his liver. He relates the fear and gratitude he feels as he stares death in the face. If ever there was a time to get to know Sacks, especially through the intimate self-portrait he paints in *On the Move*, it is now.

**The Chimp and the River: How AIDS Emerged from an African Forest**
David Quammen
*W.W. Norton & Company, February 2015*


Seasoned science writer David Quammen brings his unparalleled knack for prose to the seldom-told, or at least oft-misunderstood, ecological origin story of the HIV/AIDS epidemic. In *The Chimp and the River*—extracted from his 2012 best-seller *Spillover*, updated and with new material added—Quammen deftly reconstructs the epidemic's zoonotic origin, which likely involved a single human contracting the pandemic group M type of HIV-1 from a chimp he butchered in southeastern Cameroon around 1908. As ever, the author places himself in the action, travelling to remote corners of Africa and infectious disease labs across the world.

Quammen probes the zoonotic nature of HIV/AIDS and reminds readers that the 12 different existing groups of the virus (eight of HIV-2 and four of HIV-1) spilled over from animal host to human in a series of unfortunately common events. "HIV hasn't happened to humanity just once," he writes. "It has happened at least a dozen times—a dozen that we know of, and probably many more times in earlier history. The arrival of HIV in human bloodstreams was, on the contrary, part of a small trend."

**Domesticated: Evolution in a Man-Made World**
Richard C. Francis
*W.W. Norton & Company, May 2015*




Domestication has brought humankind into close proximity with erstwhile wild animals that for millennia have provided us with companionship, food, protection, hunting help, and fibers. But how we tamed free-living creatures to the point that they became amenable to confinement, servitude, and/or snuggling is largely lost to history. Science journalist Richard Francis, who also has a PhD in neurobiology, draws on a variety of scientific disciplines to construct case studies for a number of different species in *Domesticated: Evolution in a Man-Made World*.

Each chapter of the book's first half highlights the evolutionary transformation of one species into its domestic form. Francis tackles dogs, cats, ferrets, mink, pigs, cattle, sheep, goats, reindeer, camels, horses, mice, and rats, among other human-proximal animals. Although the archaeological, anthropological, genomic, and evolutionary story differs for each case of domestication, a couple of common threads emerge: virtually all domestic breeds retain genetic traces of their wild forebears, and domestication often results in the selection for and retention of juvenile traits. In the latter half of *Domesticated*, Francis trains his sights on human evolution, emphasizing how that process was aided by the subjugation of our fellow animals. "Domestication is an example of how our dominion is less a matter of adaptation, as biologists typically understand the term," he writes in the book's final chapter, "and more a matter of adapting our environment to our own ends."

—Bob Grant

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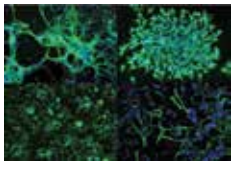

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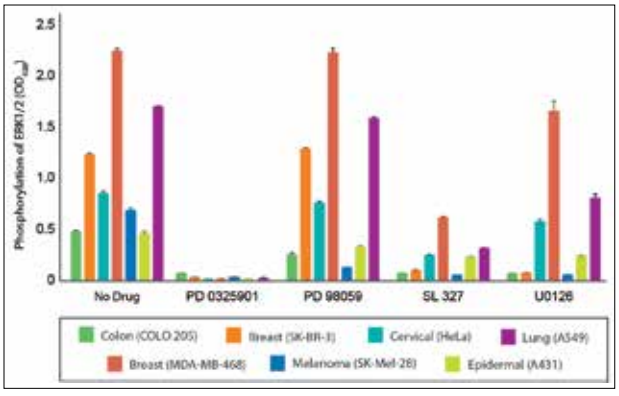


*Figure*: The Effectiveness of Four MEK Inhibitors in Seven Cancer Cell Lines was Assessed using R&D Systems ELISAs.

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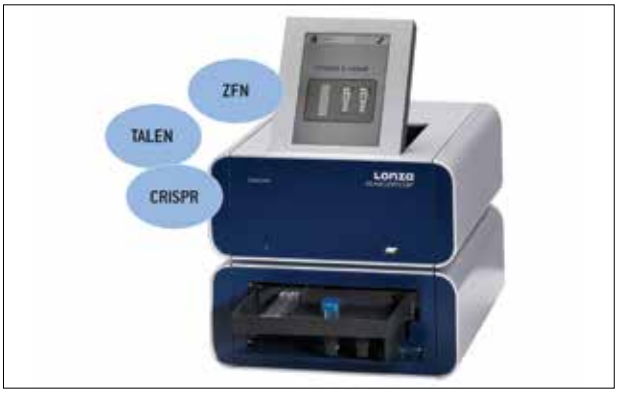


Genome editing enables the targeted genetic engineering of a cell's DNA, making it a powerful tool across a range of applications, from fundamental biological research and drug discovery through to agriculture and bioproduction. The process utilizes sequence specific nucleases to delete, insert or replace a designated sequence in a target genome. Three main systems are commonly used: zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and the CRISPR/Cas9 nuclease system. In all cases, the nucleases generate double-stranded genomic DNA breaks, which are then repaired via either non-homologous end-joining (which is used to generate deletions) or homology dependent repair (which is used to replace a gene or generate new insertions).

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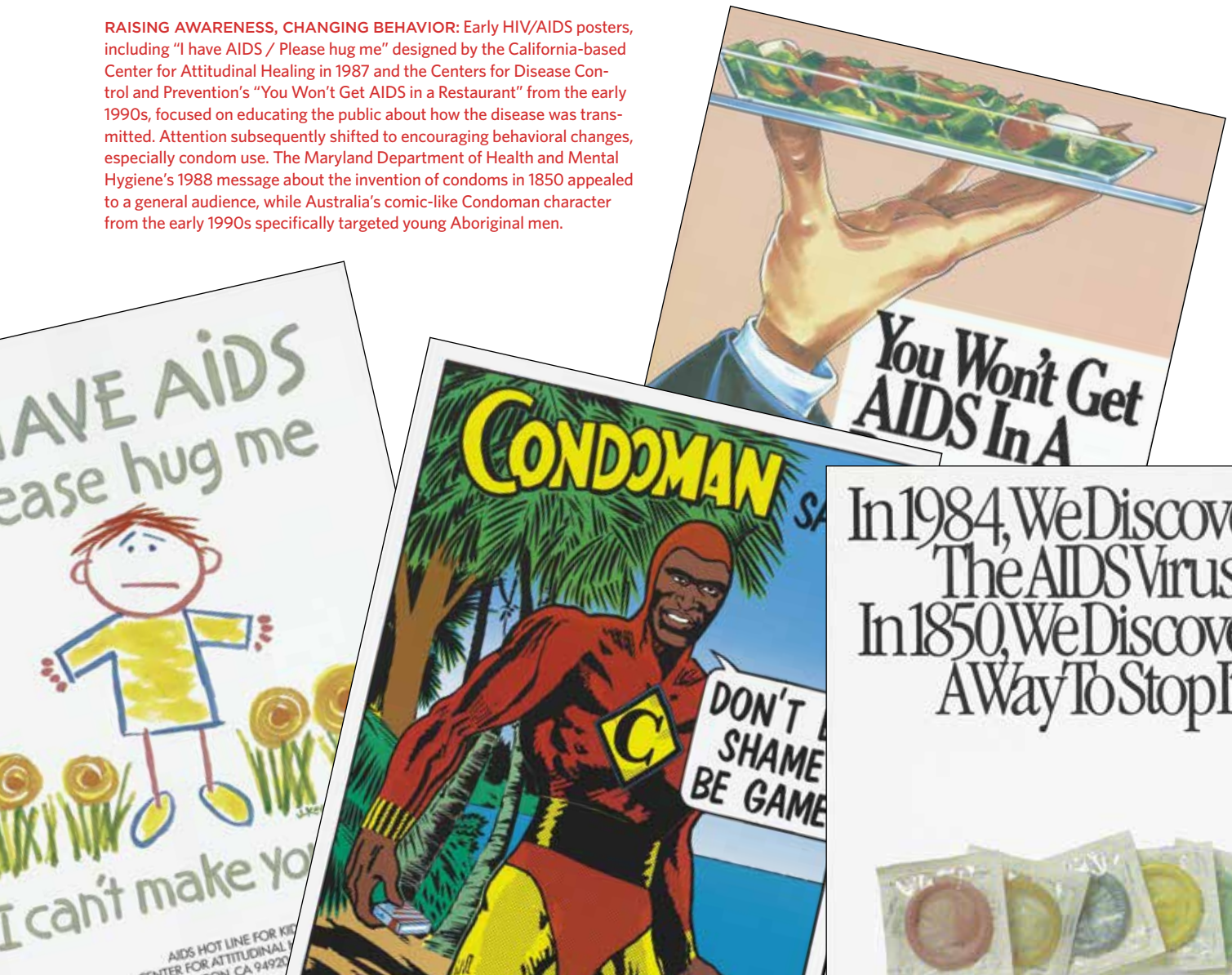
# Early AIDS Messages

BY JENNY ROOD

In 1985, 95 percent of Americans had heard of AIDS, and 47 percent admitted they would prefer to avoid HIV-positive people, according to a Kaiser Family Foundation report. The following year, to promote the scientific knowledge detailed in the Surgeon General's new report on the disease, the American Red Cross and the Ad Council began the first of many public-education campaigns, followed by the US government's America Responds to AIDS effort, which lasted from 1987 until 1996.

Originally appearing in print ads, on billboards, and on public transportation, the posters—humble messengers of the multimedia campaign—serve today as reminders of how public understanding of the disease progressed. The earliest images fought misinformation about disease transmission. Later, the focus shifted from raising awareness to changing risky behavior by encouraging condom use and discouraging the sharing of hypodermic needles. Messaging also began to target specific groups—such as gay men and minority communities—that were hardest hit by the virus. For example, Australia's Condoman character merged a masculine, muscled superhero with a safe-sex message to dispel the idea, common among young Aboriginal men, that condom use was unmanly.

Because they represent the key messages of the earliest eras of HIV/AIDS education, "these posters are classic," says Seth Noar, a professor of health communication at the University of North Carolina at Chapel Hill. "[They] likely played a role in both increasing knowledge about HIV and then later trying to impact behavior," he says, adding that it's hard to imagine seeing similar posters—with what are now such obvious, well-known facts—these days. ■

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**RAISING AWARENESS, CHANGING BEHAVIOR:** Early HIV/AIDS posters, including "I have AIDS / Please hug me" designed by the California-based Center for Attitudinal Healing in 1987 and the Centers for Disease Control and Prevention's "You Won't Get AIDS in a Restaurant" from the early 1990s, focused on educating the public about how the disease was transmitted. Attention subsequently shifted to encouraging behavioral changes, especially condom use. The Maryland Department of Health and Mental Hygiene's 1988 message about the invention of condoms in 1850 appealed to a general audience, while Australia's comic-like Condoman character from the early 1990s specifically targeted young Aboriginal men.

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